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Increased risk of lung cancer associated with a functionally impaired polymorphic variant of the human DNA glycosylase NEIL2

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ABSTRACT

Human NEIL2, one of five oxidized base-specific DNA glycosylases, is unique in preferentially repairing oxidative damage in transcribed genes. Here we show that depletion of NEIL2 causes a 6–7-fold increase in spontaneous mutation frequency in the *HPRT* gene of the V79 Chinese hamster lung cell line. This prompted us to screen for NEIL2 variants in lung cancer patients' genomic DNA. We identified several polymorphic variants, among which R103Q and R257L were frequently observed in lung cancer patients. We then characterized these variants biochemically, and observed a modest decrease in DNA glycosylase activity relative to the wild type (WT) only with the R257L mutant protein. However, in reconstituted repair assays containing WT NEIL2 or its R257L and R103Q variants together with other DNA base excision repair (BER) proteins (PNKP, Polβ, Lig IIIα and XRCC1) or using NEIL2-FLAG immunocomplexes, an ~5-fold decrease in repair was observed with the R257L variant compared to WT or R103Q NEIL2, apparently due to the R257L mutant's lower affinity for other repair proteins, particularly Polβ. Notably, increased endogenous DNA damage was observed in NEIL2 variant (R257L)-expressing cells relative to WT cells. Taken together, our results suggest that the decreased DNA repair capacity of the R257L variant can induce mutations that lead to lung cancer development.

1. Introduction

Lung cancer is the leading cause of cancer-related death in both men and women, claiming ~1.4 million lives globally (WHO) and 157,300 in the United States in 2010 [1]. In most cases, tumors are detected at advanced stages, and the overall 5-year survival rate is only 15%. Lung cancer is broadly classified as either non-small-cell

Abbreviations: AP site, apurinic/apyrimidinic site; BER, base excision repair; Fpg, formamidopyrimidine [fapy]-DNA glycosylase; HPRT, hypoxanthine phosphoribosyltransferase; IC, immunocomplex; LA QPCR, long amplicon quantitative PCR; Lig IIIα, ligase IIIα; MAF, minor allele frequency; NEIL2, Nei-like 2; NSCLC, non-small-cell lung carcinoma; NTH1, endonuclease III homolog 1; OGG1, 8-oxoguanine-DNA glycosylase; OR, Odd ratio; PLA, proximity ligation assay; PNKP, polynucleotide kinase 3′-phosphatase; Polβ, polymerase β; RFLP, restriction fragment length polymorphism; RNAPII, RNA polymerase II; SCLC, small-cell lung carcinoma; SNP, single nucleotide polymorphism; SSBs, single-strand breaks; TC-BER, transcription-coupled BER; 6-TG, 6-thioguanine; UTR, untranslated region; WT, wild type; XRCC1, X-ray repair cross-complementing protein 1.

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(NSCLC) or small-cell carcinoma (SCLC) [2,3]. NSCLC, which constitutes ~80% of all cases, grows more slowly; SCLC grows quickly and is always caused by smoking. NSCLC has 3 subtypes: adeno, squamous cell and large-cell carcinoma. Lung cancer is primarily an environmental disease, with cigarette smoke being the primary cause. Cigarette smoke contains thousands of chemicals, many of which are known carcinogens. Exposure to radon gas is another major cause of lung cancer. Radon is an alpha particle emitter; when inhaled, their decay will target cells in the respiratory epithelium, causing damage to their genetic material [4,5]. One of the very early steps in carcinogenesis due to exposure to these hazards is the generation of reactive oxygen species (ROS). ROS-induced mutations are a known prerequisite for many diseases, particularly cancer [6–8].

ROS-induced oxidation of DNA is complex, leading to single-strand breaks (SSBs) and a multitude of modifications to DNA bases, many of which are highly mutagenic and/or toxic [9,10]. Oxidized base lesions in DNA are repaired via a highly conserved multistep process, the base excision repair (BER) pathway that is initiated by excision of the damaged base by a member of a class of enzymes called DNA glycosylases [11–13]. Five oxidized base-specific DNA glycosylases have been identified and characterized so far in human cells. 8-Oxoguanine-DNA glycosylase (OGG1) and endonuclease III homolog 1 (NTH1) were characterized initially and shown to preferentially excise oxidized purines and pyrimidines, respectively [14,15]. Several years later we and others identified NEIL (Nei-like) 1–3, whose products share conserved motifs with *E. coli* MutM or Nei [16–20] and excise both purine and pyrimidines oxidation products. The NEILs are distinct from NTH1 and OGG1 both in their structural features and reaction mechanisms [16,17]. Upon recognition of an oxidized base, the N-glycosylic bond is cleaved by a DNA glycosylase, releasing the free base, followed by cleavage of the phosphodiester backbone by an associated AP lyase activity, which leaves a blocked 3′-terminus in the resulting nick [21,22]. The block is removed by the 3′ end-cleaning activity of either AP endonuclease or polynucleotide kinase 3′ phosphatase (PNKP) [23–25]. We have shown that OGG1/NTH1-initiated repair is dependent on APE1, whereas NEIL1- and -2-mediated repair involves PNKP [25,26]. The resulting gap in the lesion-containing strand is then filled in by DNA polymerase. Finally, the remaining nick in the repaired strand is sealed by DNA ligase [13,27].

Unlike OGG1 and NTH1, which are active only with duplex DNA, NEIL1 and NEIL2 excise lesions from DNA bubble structures or single-stranded (ss) DNA [28], which are generated transiently during DNA replication and transcription. Our recent studies have indicated that NEIL1 is primarily involved in the repair of replicating genomes [29,30], and that NEIL2 primarily removes the oxidized bases from transcribing genes via transcription-coupled BER (TC-BER) [31]. This repair pathway is particularly important in terminally differentiated non-dividing cells, the majority of cells in adult mammals. In these cells, mutation fixation by replication is not a concern, so only repair of the transcribed strand in functional genes is necessary to maintain a functional transcriptome and to prevent the synthesis of mutant RNAs and proteins [32,33].

We thus reasoned that a deficiency in NEIL2-dependent repair pathways would have severely deleterious consequences. Based on this hypothesis, we examined the consequences of NEIL2 depletion in the V79 Chinese hamster lung cell line and observed a significant increase in spontaneous mutation frequency in those cells, implicating NEIL2 in preventing mutations linked to carcinogenesis. This observation prompted us to screen for NEIL2 genetic variations in the genomic DNA from lung cancer patients. We identified two such variants that were common in lung cancer patients. One of these variants had reduced association with NEIL2s interacting partners for BER, resulting in inefficient repair of oxidized bases and accumulation of endogenous genomic damage. Based on these results we propose that persistent genomic damage due to functional deficiency of this NEIL2 variant could contribute to lung carcinogenesis.

2. Materials and methods

2.1. Study subjects

For our initial study (Table 1), we obtained genomic DNA from 20 lung cancer patients (European-American) from The Human Tumor Bank Core at UTMB. We then received additional samples of 99 European-American and 52 Chinese-American lung cancer patients from the City of Hope (CA). Control genomic DNA from 200 healthy individuals (European-American) was purchased from Sigma.

For studies involving the Chinese population (Tables 2–4 and Supplementary Table 1), the case-control study consisted of 670 patients with lung cancer and 666 population controls. All subjects were ethnic Han Chinese living in Beijing and the surrounding regions. Patients were recruited from January 2005 to January 2007 at the Cancer Hospital, Chinese Academy of Medical Sciences (Beijing, China). The response rate of patients was 100%. Control subjects were cancer-free individuals living in the same region as the cancer patients, with a 96% response rate. The cases and controls were frequency-matched by sex and age.

2.2. Genotyping

For genotyping of samples available from UTMB and the City of Hope, all four NEIL2 exons were PCR-amplified and sequenced in UTMB's core facilities. Electropherograms were aligned with STADEN software to identify the mutations. However, for the Chinese population genomic DNA was extracted from blood samples. The classical PCR-based RFLP method was used to genotype (rs8191664) the R257L variant. The forward and reverse primers for PCR were 5′-CCCCGCTTTATTTCAAGGAACATCATT-3′ and 5′-CACCACGTGATCCACTAGGACCTGC-3′, respectively, yielding a product of 123 bp. A 10 μl reaction mixture was used comprising 100 ng DNA, 0.2 μM each primer, 0.3 mM each deoxynucleotide triphosphate, 2.0 mM $MgCl_2$, and 0.5 units of Taq DNApolymerase

Table 1
SNP analysis in NEIL2 in lung cancer patients.

Exon	SNP ID	NT changes	AA changes	Cases (EA, $n=119$) n (%, MAF)	Controls (EA, $n=200$) n (%, MAF)	Case (CHA, $n=52$) n (%, MAF)	(database, dbSNP) MAF	
							CEU	Chinese
2	NA	A>T	H12L	1 (0.8, 0.004)	–	–	–	–
3	NA	G>A	E77K	1 (0.8, 0.004)	–	–	–	–
3	rs8191613	G>A	R103Q	6 (5, 0.025)	3 (1.5, 0.0075)	25 (48, 0.24)	0.017	0.22
4	rs8191666	A>C	P123T	1 (0.8, 0.004)	–	–	0.0013	–
5	rs8191664	G>T	R257L	10 (8, 0.04)	4 (2, 0.01)	26 (50, 0.25)	0.013	0.25

NT, nucleotide; AA, amino acid; EA, European-American; CHA, Chinese-American; NA, not applicable; MAF, minor allele frequency; (–) not available; CEU, Caucasian.