Carcinogenesis vol.15 no.12 pp.2769–2773, 1994

Loss of heterozygosity of the human cytosolic glutathione peroxidase I gene in lung cancer

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The consistent deletion of 3p21 in lung cancer has led to intensive efforts to identify a lung tumor suppressor gene at this locus. We recently mapped the gene for the selenium-dependent drug-detoxifying enzyme glutathione peroxidase 1 (*GPX1*) to this location by *in situ* hybridization. We developed a polymerase chain reaction-based assay which demonstrated the existence of three *GPX1* alleles characterized by the number of alanines in a polyalanine coding sequence in exon 1. These three alleles produced a heterozygote frequency of 70% in two separate populations: normal tissue DNA taken from Centre d'Etude du Polmorphisme Humain (CEPH) parents and normal tissue taken from cancer patients. In contrast, 10 heterozygote tumors were detected out of 64 lung cancer specimens. Linkage analysis of *GPX1* to Genethon 3p markers in CEPH pedigrees demonstrated that *GPX1* was located between the two microsatellite markers believed to flank the lung cancer deletion site. Nucleotide sequence analysis of *GPX1* alleles did not reveal any mutations of this gene in lung tumors. However, sequence analysis did reveal that the three *GPX1* alleles were characterized by three nucleotide substitutions in addition to the polyalanine polymorphism, including a substitution at codon 198 which results in either a proline or leucine at that position. Therefore, the different *GPX1* alleles encode structurally different hGPx1 subunits. In addition, analysis of allele frequency suggests that the *GPX1*ALA7* allele may occur less frequently in tumors with 3p21 deletions.

Introduction

Lung cancer epidemiology has demonstrated environmental associations with both its prevalence and prevention. Cigarette smoking, with its resultant exposure to DNA-damaging activated hydrocarbon products, is estimated to be the cause of 85–90% of all lung cancers (1). Dietary selenium, on the other hand, has been implicated in the chemoprevention of lung cancer (2,3). In addition, cytogenetic analysis of lung tumors has determined consistent chromosomal abnormalities in these tumors. Virtually all small cell lung cancers (SCLCs*) and 50% of non-small cell lung cancers (NSCLCs) contain a deletion of the short arm of chromosome 3, in the region of 3p21, by karyotype analysis and loss of heterozygosity studies (4–10). Although cigarette smoking is associated with increased damage at fragile chromosomal sites in peripheral blood and bone marrow cells (11,12), the exact relationship of the environmental factors to the genetic abnormalities which result in lung cancer is not yet understood.

Human cellular glutathione peroxidase I (hGPx1) is a selenium-dependent enzyme that participates in the detoxification of activated oxygen species by catalyzing reduction of these damaging compounds through the coupled oxidation of reduced glutathione (13). We have recently reported that the *GPX1* locus maps to chromosome 3p21 by *in situ* hybridization analysis of lymphocyte metaphase spreads (14), and that *GPX1* is immediately downstream from another gene previously mapped to 3p21, *RHOA*. (15). We also found that a cloned *GPX1* gene contained a sequence polymorphism, with a variable number of GCG triplet nucleotide repeats encoding for alanine in a polyalanine tract in exon 1 (15).

The presence of an informative polymorphism in the *GPX1* locus would provide a genetic tool with which to look for hemizygous or homozygous *GPX1* deletions in lung cancer. We therefore developed a PCR-based assay to detect and characterize the polyalanine polymorphism. Analysis of this region demonstrated the existence of three alleles with an observed heterozygosity in 70% of normal tissue specimens analyzed. Thus, we were able to use this assay both to locate the *GPX1* locus relative to other 3p markers by linkage analysis with pedigrees provided by the Center d'Etude du Polymorphism Humain (CEPH) and to determine the frequency with which this locus was lost in lung cancer.

Materials and methods

Polymorphism detection

Lung cancer and renal cell carcinoma samples were either primary cell lines derived from tumors or surgical specimens as previously described (16–18). Paired normal tissue DNA was extracted from patient lymphocytes. Between 50 and 100 ng of cellular DNA was amplified by polymerase chain reaction (PCR) using standard methods. Custom oligonucleotide primers were purchased from the Midland Certified Reagent Company (Midland, TX). The 5′ primer, starting at −61 relative to the start of transcription, was GAAACTGCCTGTGCCACGTGACC (Figure 1). The 3′ primer was CGAGAAGGCATACACCGACTGGGC, which started from +107 relative to the start of transcription of the cloned *GPX1* sequence (Figure 1). Therefore,

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                                                    5'
-90   GGCGGATGAGGCGGGACCCTCAGGCCCGGAAAACTGCCTGTGCCACGTGACCCGCCGGCC
                                                   +1
-30   AGTTAAAAGGAGGCGCCTGCTGGCCTCCCCCTATAGTGCTTGTTCGGGGCGCTCCGCTGGC
-31   TTCTTGGACAATTGCGCC  ATG TGT GCT GCT CGG CTA GCG GCG GCG GCG
                          met cys ala ala arg leu ala ala ala ala
                                                    3'
+80   GCG GCC CAG TCG GTG TAT GCC TTC TCG GCG CGC CCG CTG GCC GGC
      ala ala gln ser val tyr ala phe ser ala arg pro leu ala gly
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Fig. 1. Nucleotide sequence of the 5′ flanking sequence, the 5′ untranslated region (UTR) and the 5′ coding region of *GPX1*. Underlined sequences show the position of primers used for PCR amplification of the segment of DNA containing the polyalanine polymorphism (in italics). The sequence shown is for an allele containing six alanines.

*Abbreviations: SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; hGPx1, the human cytosolic glutathione peroxidase 1 enzyme; *GPX1*, the gene encoding the human glutathione peroxidase 1 enzyme; CEPH, Centre d'Etude du Polmorphisme Humain; PCR, polymerase chain reaction; LOH, loss of heterozygosity; UTR, untranslated region.

alleles containing five, six or seven alanines in this tract would produce PCR fragments of 165, 168 or 171 nucleotides in length respectively. The 3′ primer was end-labeled with [γ-^{32}P]ATP with T4 polynucleotide kinase (Promega) and the reactions were performed in a Perkin Elmer 9600 thermal cycler with an annealing temperature of 60°C for 30 cycles. The PCR products were size-fractionated on a 6% polyacrylamide sequencing gel and detected by autoradiography.

Linkage analysis

Human genomic DNA samples from CEPH families 102, 104, 884, 1331, 1332, 1347, 1362, 1413 and 1416 were used for the linkage analysis. This subset of the CEPH panel was composed of the nine largest families and consisted of 146 individuals. The microsatellite-based genetic linkage map constructed by Jean Weissenbach and co-workers (21) was based on genotyping of these CEPH families. The *GPX1* locus genotyping data for these reference families were entered into CEPH database version 6 and converted to a format suitable for use with the CRIMAP program (19, 20) using LNKTOCRI (prepared by J.Reefer and A.Chakravarti and obtained from Ken Buetow). Two-point and multipoint linkage analyses were performed with CRIMAP version 2.4 on a VAX/VMS mainframe computer.

The Genethon microsatellite markers for chromosome 3p described by Weissenbach (21) were used for mapping the *GPX1* locus. Genotyping data deposited in the CEPH database for 10 uniquely ordered, highly polymorphic Genethon markers and data from the additional microsatellite D3S1358 (22), were used to construct a linkage map with an order supported by odds greater than 1000:1. The *GPX1* locus was added to these loci using the option BUILD. The FLIPS option examined the permutations of two or three loci in order to detect incorrect positions of adjacent loci. The Kosambi interference function was used to calculate genetic distance.

Sequence analysis

Both exons of *GPX1* in 16 lung tumor specimens were amplified by PCR using primers which spanned the entire coding region. The *GPX1* 5′ flanking sequence in seven lung tumor specimens was amplified by primers which spanned the 802 bp sequence between *RHOA* and *GPX1*. After treatment with a DNA purification system (Promega) the nucleotide sequence of the PCR fragments was determined using primers end-labeled with [γ-^{33}P]ATP and T4 DNA polynucleotide kinase (Promega). The sequencing reactions were performed in a Perkin Elmer 9600 thermal cycler using a thermal cycle sequencing kit (Stratagene) according to the manufacturer's instructions and size-fractionated on a 6% polyacrylamide gel. Nucleotide substitutions characteristic of the three *GPX1* alleles were confirmed by sequencing those regions in an additional 21 specimens.

Results

We first determined the size distribution of the polyalanine tracts, the relative frequency of each allele in a normal population and the percentage of normal genotypes that were heterozygous. As shown in Table I, we found evidence for only three alleles, with five, six and seven alanines in the polyalanine sequence. Each allele occurred with significant frequency, and ~70% of genotypes were heterozygous in two different normal populations: the European CEPH parents and normal tissue taken from American cancer patients.

Table I. Observed heterozygote frequency of GPX1 polyalanine polymorphism

	CEPH parents (n = 52)	Normal tissue from cancer patients (n = 42)
Homozygotes		
*GPX1*ALA5, GPX1*ALA5*	4	4
*GPX1*ALA6, GPX1*ALA6*	5	7
*GPX1*ALA7, GPX1*ALA7*	7	1
Total	16	12
% observed homozygotes	30%	29%
Heterozygotes		
*GPX1*ALA5, GPX1*ALA6*	11	13
*GPX1*ALA5, GPX1*ALA7*	18	14
*GPX1*ALA6, GPX1*ALA7*	7	3
Total	36	30
% observed heterozygotes	70%	71%

Since we identified a polymorphism that thus appeared to have the possibility of being informative in 70% of cases, we examined SCLC, NSCLC and non-papillary renal cell carcinoma (RCC) cell lines and tumors for the presence of *GPX1* alleles. As summarized in Table II, in SCLC, a tumor virtually always associated with 3p21 deletions, none of 20 specimens (14 cell lines and 6 tumors) were heterozygous at the *GPX1* locus. In comparison, in a series of 44 NSCLCs, a tumor that is associated with 3p21 deletions in about half the cases, 10 heterozygous tumors were detected. In non-papillary RCC, a tumor associated with 3p21 deletions, 2/27 RCC specimens were heterozygous. DNA from normal tissue was available from 13 lung cancer patients and 24 patients with renal cell carcinoma. Of the 13 lung cancer patients, nine were informative (heterozygous) and all nine had tumors which showed loss of heterozygosity (LOH) for *GPX1* (six SCLC and three NSCLC). Of the 24 renal cell carcinoma patients, 19 were informative and 17/19 (89%) showed LOH at *GPX1*. We also examined normal and tumor cell line DNA from six patients with papillary renal cell carcinomas, a tumor not associated with 3p deletions. Of these six specimen pairs, three were informative, and none of the three showed LOH for *GPX1*.

To determine whether *GPX1* was located between microsatellite markers believed to flank the site of deletions in lung cancer, we used *GPX1* polymorphisms in CEPH pedigrees to map the location of *GPX1* relative to microsatellite markers (Table III). The *GPX1* locus was placed on the Genethon microsatellite chromosome 3p map between D3S1260 and D3S1289 with the likelihood odds of at least 1000:1. D3S1358, a tetranucleotide repeat developed by one of us (22), also uniquely localized between these markers, distal to the *GPX1* locus. Table III shows the recombination frequencies, Kosambi distances and local support for the *GPX1* locus and the other 11 microsatellite markers on the Genethon chromosome 3p

Table II. Observed *GPX1* heterozygote frequency in lung cancer and renal cell carcinoma

	SCLC	NSCLC	RCC
Tumors examined	20	44	27
Heterozygotes	0	10	2
Normal tissues examined	9	4	24
Informative cases	6	3	19
LOH/Informative cases	6/6	3/3	17/19

Table III. Recombination frequencies (q), Kosambi distances (cM) and local support for the *GPX1* locus and 11 uniquely ordered microsatellite markers on the sex-averaged genetic linkage map of chromosome 3p

Locus	θ	cM	Local support
D3S1293	–	–	–
D3S1266	0.08	7.8	6.92
D3S1298	0.10	9.7	21.11
D3S1260	0.02	2.0	1.79
D3S1358	0.07	7.2	21.11
GPX1	0.01	1.0	2.95
D3S1289	0.01	0.8	3.03
D3S1295	0.04	4.4	6.33
D3S1300	0.04	3.9	9.52
D3S1312	0.03	2.7	3.48
D3S1287	0.07	6.8	7.15
D3S1285	0.01	1.2	1.26

Local support is the $\log_{10}$ likelihood difference when adjacent pairs of loci were reversed by the FLIPS option of CRIMAP.

map. The Genethon markers D3S1260 and D3S1289 were identified by Naylor *et al.* to define the region of minimal deletion in lung cancer (23).

We examined the nucleotide sequence of the *GPX1* open reading frame from 17 lung tumors and found no evidence for *GPX1* mutations. We also found no mutations in these specimens in the intron−exon borders or in a sequence in the 3′ untranslated region recently shown to be important for insertion of the rare seleno-cysteine amino acid residue in the nascent polypeptide (24). In addition, we examined the 802 bp 5′ flanking sequence between *RHOA* and *GPX1* in seven lung tumors and found no mutations in this region.

Nucleotide sequence analysis did reveal, however, that other nucleotide substitutions co-segregate with the polyalanine polymorphism. The characteristics of these alleles are summarized in Table IV. The *GPX1* alleles with five or seven alanines are identical except for the length of the polyalanine polymorphism. The *GPX1* allele with six alanines in the polyalanine sequence, *GPX1*ALA6*, differs from the others in that it has a leucine for proline substitution at codon 198, a T for C substitution at +2, and a G for A substitution at −592.

Therefore, since the different *GPX1* alleles encode structurally different hGPx1 subunits, we determined whether there was a difference in the occurrence of the different *GPX1* alleles in lung cancer and RCC. We compared tumors associated with deletion of the lung cancer locus at 3p21 (SCLCs and homozygous NSCLCs) to closely associated tumors that do not have deletions at the lung cancer deletion site (heterozygous NSCLCs and RCCs). As shown in Table V, there was a trend toward decreased frequency of the *GPX1*ALA7* allele in homozygous versus heterozygous NSCLC specimens (18% versus 35% respectively) that was not statistically significant (comparing frequency of alleles *GPX1*ALA5* and *GPX1*ALA6* versus *GPX1*ALA7*, chi square $P = 0.19$) and the same trend in the comparison of SCLC specimens to non-papillary RCCs (15% versus 28% respectively).

Discussion

Lung cancer is a malignancy directly caused by exposure to smoking-related carcinogens (1). In contrast, dietary selenium has been associated with a decreased risk of lung cancer (2,3). The gene for the selenium-dependent drug-detoxifying glutathione peroxidase 1 enzyme, *GPX1*, was originally mapped to 3q11–13.1 (25). However, we isolated a genomic fragment that contained *GPX1* and the 3′ end of another gene, the Ras-related gene *RHOA*, in its 5′ flanking sequence (15). The gene encoding *RHOA* had been mapped to 3p21 (26), and we recently confirmed that both genes map to 3p21 by *in situ* hybridization (14). In this report we demonstrate that the gene for the selenium-dependent hGPx1 enzyme is located within the minimal region of 3p21 that is frequently deleted in lung cancers, and that LOH of *GPX1* was observed in all informative cases of lung cancer examined.

Glutathione peroxidases protect cells against oxidative damage by reducing hydrogen peroxide and a wide range of organic peroxides with reduced glutathione (13). Glutathione peroxidase activity has been detected in lung tumors (27,28), but it is unclear which glutathione peroxidase isozymes account for this activity. The classic cytosolic form of the enzyme, hGPx1, belongs to a family of selenium-dependent glutathione peroxidases which includes another cytosolic form of the enzyme hGPx2 (29), a plasma glutathione peroxidase hGPx3 (30) and a phospholipid hydroperoxide glutathione peroxidase hGPx4 (31). hGPx2, hGPx3 and hGPx4 have been localized to chromosomes 14, 5 and 19 respectively (32).

Several *in vitro* and *in vivo* studies have shown that selenium exposure increases GPx1 activity and RNA expression, although not *GPX1* gene transcription (15,33–37). Numerous case-control epidemiological studies have examined the relationship of serum selenium levels and the risk of lung cancer (38–42). The results of these studies have been mixed, possibly because of small sample sizes and the inherent variability of serum selenium levels. Recently, however, measurement of toenail selenium levels has been validated as a reliable measure of long-term selenium intake (43–45), and a large prospective study of over 120 000 men and women has demonstrated an inverse association between toenail selenium levels and the risk of lung cancer. These epidemiological findings are supported by studies which demonstrate that selenium can prevent lung tumor induction by i.p. injection of the tobacco-specific toxin 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (46). In this paper we have demonstrated that a polymorphism exists in the coding region of *GPX1* which gives rise to a significant (70%) observed frequency of heterozygosity in two normal populations (Table I). In contrast, no heterozygosity was observed in the *GPX1* locus in SCLC, suggesting LOH of this locus in this disease (Table II). In the series of 44 NSCLCs, we would have expected to find ~15 heterozygotes (half of the NSCLCs would lack 3p21 deletion and 70% of these would occur in heterozygotes). We observed heterozygosity in 10/44 NSCLCs, indicating that LOH at this locus does not occur at a significantly greater frequency than suggested by previous cytogenetic analyses.

Nucleotide sequence analysis demonstrated that the three *GPX1* alleles identified by the polyalanine polymorphism contained other characteristic nucleotide substitutions, including a substitution which results in an amino acid substitution at the carboxy end of the hGPx1 subunit (Table IV). We also found a trend in the frequency of the *GPX1*ALA7* allele between homozygous lung tumors and two groups of closely related tumors: RCCs, tumors associated with 3p deletions but not at the lung cancer locus, and heterozygous NSCLCs. Since these tumor specimens came from patients accrued from the same population base, ethnic or geographic variation should not account for differences in these allele frequencies. Clearly,

Table IV. Characteristics of *GPX1* alleles

Allele	nt at −592	nt at +2	No. of alanines	Codon 198
*GPX1*ALA5*	A	C	5	CCC (proline)
*GPX1*ALA6*	G	T	6	CTC (leucine)
*GPX1*ALA7*	A	C	7	CCC (proline)

Table V. Frequency of *GPX1* alleles in SCLC, NSCLC and RCC

Allele[a]	NSCLC heterozygotes (n = 20)	NSCLC homozygotes (n = 34)	RCC (n = 29)	SCLC (n = 20)
*GPX1*ALA5*	6 (30%)	18 (53%)	11 (38%)	6 (30%)
*GPX1*ALA6*	7 (35%)	10 (29%)	10 (34%)	11 (55%)
*GPX1*ALA7*	7 (35%)	6 (18%)	8 (28%)	3 (15%)

[a]Tumors are assumed to contain only one allele if only a single allele is detected.
n = number of alleles.

a larger study population will be needed to verify these preliminary findings.

No mutations were found within the remaining *GPX1* alleles from lung tumors. Therefore, *GPX1* does not have the characteristics expected of a classic tumor suppressor gene. However, the findings in this study are compatible with the possibility that certain constitutional *GPX1* genotypes may increase the risk of developing lung cancer, or that deletion of a *GPX1* allele may result in lung cancer by increasing susceptibility to carcinogens. It is also possible that *GPX1* is only a bystander playing an auxillary role, providing a useful polymorphism closely linked to the lung cancer tumor suppressor gene on 3p21. Ongoing studies of*GPX1* genotypes and gene expression in lung cancer patients and their tumors, as well as biochemical characterization of the hGPx1 enzymes encoded by these distinct alleles, should help clarify the possible role of *GPX1* in lung cancer.

Acknowledgements

We thank Seth Steinberg, Ph.D., for his help in the statistical analysis of the data.

References

1. Greenwald,P. (1985) Prevention of Cancer. In DeVita,V.T., Hellman,S.A. and Rosenberg,S.A. (ed.), *Cancer Principles and Practice of Oncology*. J.B. Lippincott, Philadelphia, pp. 197–214.
2. Knekt,P., Aramaa,A., Maatel,J., Alfthan,G., Aaran,R.K., Hakama,M., Hakulinen,T., Peto,R. and Teppo,L. (1990) Serum selenium and subsequent risk of cancer among Finnish men and women. *J. Natl. Cancer Inst.*, **82**, 864–868.
3. van den Brandt,P.A., Goldbohm,R.A., van't Veer,P., Bode,P., Dorant,R.J.J., Hermus,R.J. and Sturmans,F. (1993) A prospective cohort study on selenium status and the risk of lung cancer. *Cancer Res.*, **53**, 4860–4865.
4. Whang-Peng,J., Knutsen,T., Gazdar,A., Steinberg,S.M., Oie,H., Linnoila,I., Mulshine,J., Nau,M. and Minna,J.D. (1991) Nonrandom structural and numerical chromosome changes in non-small-cell lung cancer. *Genes Chromosomes Cancer*, **3**, 168–188.
5. Yokoyama,S., Yamakawa,K., Tsuchiya,E., Murata,M., Sakiyama,S. and Nakamura,Y. (1992) Deletion mapping on the short arm of chromosome 3 in squamous and adenocarcinoma of the lung. *Cancer Res.*, **52**, 873–877.
6. Brauch,H., Johnson,B., Hovis,J., Yano,T., Gazdar,A., Pettengill,O.S., Graziano,S. and Sorenson,G.D. (1987) Molecular analysis of the short arm of chromosome 3 in small cell and non-small cell carcinoma of the lung. *New Engl. J. Med.*, **317**, 1109–1111.
7. Yokota,J., Wada,M., Shimosato,Y., Terada,M. and Sugimara,T. (1987) Loss of heterozygosity on chromosomes 3, 13, and 17 in small cell carcinoma and on chromosome 3 in adenocarcinoma of the lung. *Proc. Natl Acad. Sci. USA*, **84**, 9252–9256.
8. Naylor,S.L., Johnson,B.E., Minna,J.D. and Sakaguchi,A.Y. (1987) Loss of heterozygosity of chromosome 3p markers in small cell lung cancer. *Nature*, **329**, 451–453.
9. Johnson,B.E., Sakaguchi,A., Gazdar,A.F., Minna,J.D., Burch,D., Marshall,A. and Naylor, S.L. (1988) Restriction fragment length polymorphism studies show consistent loss of chromosome 3p alleles in small cell lung cancer patients' tumors. *J. Clin. Invest.*, **82**, 502–509.
10. Kok,K., Osinga,J., Carritt,B., Davis,M.B., van der Hout,A.H. *et al.* (1987) Deletion of a DNA sequence at the chromosome region 3p21 in all major types of lung cancer. *Nature*, **330**, 578–581.
11. Benhamou,S., Pot-Deprun,J., Sancho-Garnier,H. and Chourolinkov,I. (1988) Sister chromatid exchanges and chromosomal aberrations in lymphocytes of nurses handling cytotoxic agents. *Int. J. Cancer*, **41**, 350–353.
12. Kao-Shan,C.-S., Fine,R.L., Whang-Peng,J., Lee,E.C. and Chabner,B.A. (1987) Increased fragile sites and sister chromatid exchanges in bone marrow and peripheral blood of young cigarette smokers. *Cancer Res.*, **47**, 6278–6282.
13. Flohe,L. (1989) In Dolphin,D., Avramovic,O. and Poulson,R. (eds), *Glutathione: Chemical, Biochemical and Medical Aspects*. John Wiley, New York, pp. 644–731.
14. Moscow,J.A., He,R., Gnarra,J.R., Knutsen,T., Weng,Y., Zhao,W.-P., Whang-Peng,J., Linehan,W.M. and Cowan,K.H. (1994) Examination of human tumors for *rhoA* mutations. *Oncogene*, **9**, 189–194.
15. Moscow,J.A., Morrow,C.S., He,R., Mullenbach,G.T. and Cowan,K.H. (1992) Structure and function of the 5′ flanking sequence of the human cytosolic selenium-dependent glutathione peroxidase gene (*hgpx1*). *J. Biol. Chem.*, **267**, 5949–5958.
16. Anglard,P., Tory,K., Brauch,T.H., Weiss,G.H., Latif,F., Merino,M.J., Lerman,M.I., Zbar,B. and Linehan,W.M. (1992) Molecular analysis of genetic changes in the origin and development of renal cell carcinoma. *Cancer Res.*, **51**, 1071–1077.
17. Carney,D.N., Gazdar,A.F., Bepler,G., Guccion,J.G., Marangos,P.J., Moody,T.W., Zweig,M.H. and Minna,J.D. (1985) Establishment and identification of small cell lung cancer cell lines having classic and variant features. *Cancer Res.*, **45**, 2913.
18. Stevenson,H., Gazdar,A.F., Phelps,R. *et al.* (1990) Tumor cell lines established *in vitro*: an independent prognostic factor for survival in non-small-cell lung cancer. *Ann. Intern. Med.*, **113**, 764.
19. Donis-Keller,H., Green,P., Helms,C., Cartinhour,S. and Weiffenbach,B. (1987) A genetic linkage map of the human genome. *Cell*, **51**, 319–337.
20. Lander,E.S. and Gren,P. (1987) Construction of multilocus genetic linkage maps in humans. *Proc. Natl Acad. Sci. USA*, **84**, 2363–2367.
21. Weissenbach,J., Gyapay,G., Dib,C., Vignal,A., Morissette,J., Millasseau,P., Vaysseix,G. and Lathrop,M. (1992) A second generation linkage map of the human genome. *Nature*, **359**, 794–801.
22. Li,H., Schmidt,L., Wei,M.-H., Hustad,T., Lerman,M.I., Zbar,B. and Tory,K. (1993) Three tetranucleotide polymorphisms for loci: D3S1352; D3S1358; D3S1359. *Hum. Mol. Gen.*, **8**, 1327.
23. Naylor,S., Buys,C. and Caritt,B. (1993) Report of the Fourth International Workshop on Chromosome 3 Mapping. *Cytogenet. Cell Genet.*, **65**, 2–50.
24. Shen,Q., Chu,F.F. and Newburger,P.E. (1993) Sequences in the 3′-untranslated region of the human cellular glutathione peroxidase gene are necessary and sufficient for selenocysteine incorporation at the UGA codon. *J. Biol. Chem.*, **268**, 11463–11469.
25. Chada,S., LeBeau,M.M., Casey,L. and Newburger,P.E. (1990) Isolation and chromosome localization of the human glutathione peroxidase gene. *Genomics*, **6**, 268–271.
26. Cannizzaro,L.A., Madaule,P., Hecht,F., Axel,R., Croce,C.M. and Huebner,K. (1990) Chromosome localization of human ARH genes, a ras-related family. *Genomics*, **6**, 197–203.
27. Carmichael,J., Forrester,L.M., Lewis,A.D., Hayes,J.D., Hayes,P.C. and Wolf,C.R. (1988) Glutathione S-transferase isozymes and glutathione peroxidase activity in normal and tumour samples from human lung. *Carcinogenesis*, **9**, 1617–1621.
28. Di Ilio,C., Del Bocio,G., Cassacia,R., Aceto,A., Di Giacomo,F. and Federici,G. (1987) Selenium level and glutathione-dependent enzyme activities in normal and neoplastic human lung tissues. *Carcinogenesis*, **8**, 281–284.
29. Chu,F.-F., Doroshow,J.H. and Esworthy,R.S. (1993) Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. *J. Biol. Chem.*, **268**, 2571–2576.
30. Takahashi,K., Akasak,M., Yamamoto,Y., Kobayashi,C., Mizoguchi,J. and Koyama,J. (1990) Primary structure of human plasma glutathione peroxidase deduced from cDNA sequences. *J. Biochem.*, **108**, 145–148.
31. Maiorino,M., Chu,F.F., Paxton,R.J., Akman,S. and Doroshow,J.H. (1991) Phospholipid hydroperoxide glutathione peroxidase is the 18-kDa selenoprotein expressed in human tumor cell lines. *J. Biol. Chem.*, **266**, 7728–7732.
32. Chu,F.F. (1994) The human glutathione peroxidase genes GPX2, GPX3, and GPX4 map to chromosomes 14, 5 and 19, respectively. *Cytogenet. Cell. Genet.*, **66**, 96–98.
33. Chada,S., Whitney,C. and Newburger,P.E. (1989) Post-transcriptional regulation of glutathione peroxidase gene expression by selenium in the HL-60 human myeloid cell line. *Blood*, **74**, 2535–2541.
34. Saedi,M.S., Smith,C.G., Frampton,J., Chambers,I., Harrison,P.R. and Sunde,R.A. (1988) Effect of selenium status on mRNA levels for glutathione peroxidase in rat liver. *Biochem. Biophys. Res. Commun.*, **153**, 855–861.
35. Yoshimura,S., Takekoshi,S., Watanbe,K. and Fuji-Kuriyama,Y. (1988) Determination of nucleotide sequence of cDNA coding rat glutathione peroxidase and diminished expression of the mRNA in selenium deficient rat liver. *Biochem. Biophys. Res. Commun.*, **154**, 1024–1028.
36. Toyoda,H., Himeno,S. and Imura,N. (1989) The regulation of glutathione peroxidase gene expression relevant to species difference and the effects of dietary manipulation. *Biochim. Biophys. Acta*, **1008**, 301–308.
37. Reddy,K. and Tappel,A.L. (1974) Effect of dietary selenium and autoxidized lipids on the glutathione peroxidase system of the gastrointetinal tract and other tissues in the rat. *J. Nutr.*, **104**, 1069–1078.
38. Menkes,M.S., Comstock,G.W., Vuilleumier,J.P., Rider,A.A. and

Brookmeyer,R. (1986) Serum beta-carotene, vitamins A and E , selenium and the risk of lung cancer. *New Engl. J. Med.*, **315**, 1250–1254.
39. Willett,W.C., Morris,J.S., Pressel,S., Taylor,J.O., Polk,B.F., Stampfer,M.J., Rosner,B. and Schneider,K. (1983) Prediagnostic serum selenium and risk of cancer. *Lancet*, **2**, 130–134.
40. Salonen,J.T., Alfthan,G., Hutunen,J.K. and Puska,P. (1993) Association between serum selenium and the risk of cancer. *Am. J. Epidemiol.*, **128**, 342–349.
41. Salonen,J.T., Salonen,R., Lappetelainen,R., Maenpaa,P.H., Alfthan,G. and Puska,P. (1985) Risk of cancer in relation to serum concentrations of selenium and vitamins A and E: matched case-control analysis of prospective data. *Br. Med. J.*, **290**, 417–420.
42. Kok,F.J., van Duyn,C.M., Hofman,A., Vermeeren,R., de Bruyn,A.M. and Valkenberg,H.A. (1987) Micronutrients and the risk of lung cancer. *New Engl. J. Med.*, **316**, 1416.
43. Swanson,C.A., Longnecker,M.P., Veillon,C., Howe,M., Levander,O.M., Taylor,P.R., McAdam,P.A., Brown,C.C., Stampfer,M.J. and Willett,W.C. (1990) Selenium intake, age, gender, and smoking in relation to indices of selenium status of adults in seleniferous areas. *Am. J. Clin. Nutr.*, **52**, 859–862.
44. Hunter,D.J., Morris,J.S., Chute,C.G., Kushner,E., Colditz,G.A., Stampfer,M.J., Speizer,F.E. and Willett,W.C. (1990) Predictors of selenium concentrations in human toenails. *Am. J. Epidemiol*, **132**, 114–122.
45. Morris,J.S., Stampfer,M.J. and Willett,W. (1983) Dietary selenium in humans; toenails as an indicator. *Biol. Trace Elem. Res.*, **5**, 529–537.
46. Nakayama,T., Kaneko,M., Kodama,M. and Nagata,C. (1985) Cigarette smoke induces DNA single-strand breaks in human cells. *Nature*, **314**, 462–464.

Received on July 5, 1994; revised on September 8, 1994; accepted on September 13, 1994