

SHORT REPORT

Rare allelic imbalances, but no mutations of the *PRDX1* gene in human hepatocellular carcinomas

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J Clin Pathol 2005;58:1229–1231. doi: 10.1136/jcp.2004.024679

Allelic losses on chromosome 1p are frequent in hepatocellular carcinoma (HCC), suggesting the presence of a tumour suppressor gene in this region. The gene for peroxiredoxin 1 (*PRDX1*), an antioxidant enzyme, has been mapped to 1p34.1. Mice lacking *PRDX1* develop HCC with high frequency. Because oxidative stress has been implicated in the pathogenesis of HCC, this study was designed to determine whether the *PRDX1* gene is mutated in human HCC using loss of heterozygosity (LOH) analysis, polymerase chain reaction/denaturing gradient gel electrophoresis, and DNA sequencing. LOH of at least one of four microsatellite markers within 0.8 Mb of the *PRDX1* gene was seen in three of 34 informative HCCs, but no mutations or polymorphisms in the translated exons 2–6 of the *PRDX1* gene were found. These results suggest that genetic alterations of the *PRDX1* locus are rare events in human HCC, indicating that other genes on chromosome 1p contribute to liver carcinogenesis.

Oxidative stress caused by free radicals has been implicated in the pathogenesis of many cancers.¹ Free radicals, such as reactive oxygen species (ROS), may cause mutations in cancer related genes or directly alter the functions of proteins regulating DNA repair, cell cycle progression, and apoptosis. Oxidative stress has been linked to conditions that are associated with an increased risk of hepatocarcinogenesis, such as viral hepatitis, alcoholic liver disease, toxic liver injury, and fibrosis.^{2,3} Furthermore, hepatocellular carcinoma (HCC) shows evidence of oxidative DNA damage, as demonstrated by the formation of 8-hydroxy-2'-deoxyguanosine.^{4,5} These observations suggest that ROS induced oxidative damage contributes to the pathogenesis of HCC.

“Mice lacking *Prdx1* develop severe haemolytic anaemia and various types of malignancies, including hepatocellular carcinoma, which suggests that this protein functions as a tumour suppressor”

Several studies using karyotyping, comparative genomic hybridisation, and loss of heterozygosity (LOH) analysis have shown that loss of chromosome 1p is a recurrent genetic aberration in human HCC.⁶ Chromosomal losses most often involve the distal regions of chromosome 1p, with the 1p34–36 region involved in up to 75% of HCCs. The shortest regions of overlap on chromosome 1p have recently been mapped to 1p36.22–p36.13 and 1p36.32–p36.22.^{7,8} So far, three candidate tumour suppressor genes on the 1p36 region, the *PRDM2* (*RIZ*), *RUNX3*, and *TP73* genes, have been studied in HCC, but no mutations have been detected.^{8–10}

Peroxiredoxin 1 (*PRDX1*)—also designated MSP23, OSF-3, or PAG—is an intracellular antioxidant protein with thio-redoxin dependent peroxidase activity, which protects cells

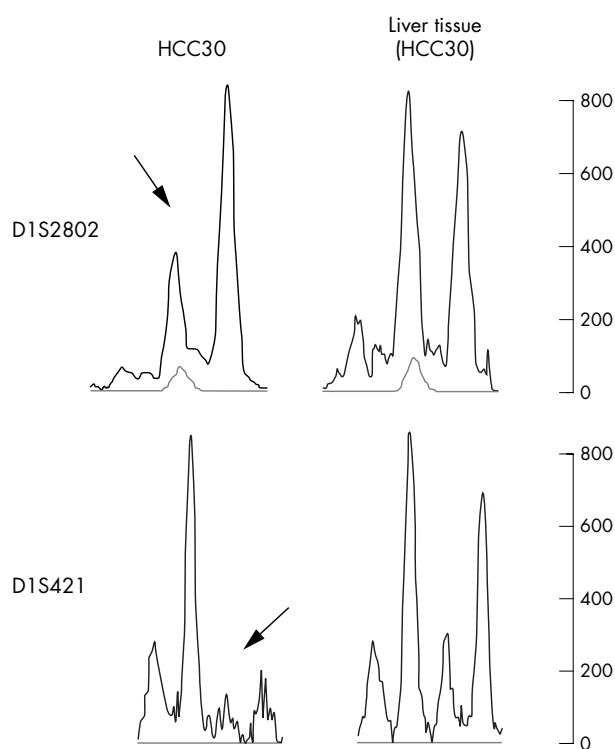


Figure 1 Loss of heterozygosity (LOH) analysis of a moderately differentiated hepatocellular carcinoma (HCC30) with allelic losses (arrows) of the microsatellite markers D1S421 and D1S2802, which are located telomeric and centromeric of the *PRDX1* locus, respectively.

against oxidative stress by inactivating ROS, and is encoded by a single gene on chromosome 1p34.1.¹¹ *PRDX1* is highly expressed in the liver and was localised to hepatocytes and Kupffer cells in the rat.^{12,13} Mice lacking *Prdx1* develop severe haemolytic anaemia and various types of malignancies, including HCC, which suggests that this protein functions as a tumour suppressor.¹⁴ However, *PRDX1* gene mutations have not been identified in human tumours.

Because distal chromosome 1p deletions are frequent in human HCC and *Prdx1* mutant mice develop HCC with high frequency, we tested the hypothesis that mutations or polymorphisms of the *PRDX1* gene may also contribute to the carcinogenesis of human HCC.

MATERIALS AND METHODS

Tumours, DNA extraction

Formalin fixed, paraffin wax embedded tumour samples of 36 HCCs and corresponding non-tumorous liver tissue were

Abbreviations: LOH, loss of heterozygosity; PCR, polymerase chain reaction; *PRDX1*, peroxiredoxin 1; ROS, reactive oxygen species

Table 1 Loss of heterozygosity analysis of the 1p34.1 region in primary hepatocellular carcinomas

Patient	Age/Sex	Aetiology	Tumour size (cm)	Grading	D1S421	D1S2802	D1S451	D1S2677	Mutation
HCC1	76/M	HCV, cirrhosis	4.2	G1	NI	ROH	ROH	NI	None*
HCC7	67/M	No cirrhosis	1.7	G2	NI	ROH	NI	ROH	None*
HCC8	55/F	HBV, cirrhosis	5.5	G2	NI	ROH	NI	ROH	None*
HCC9	51/F	HBV, fibrosis	2.4	G3	NI	ROH	NI	ROH	None*
HCC10	80/F	HCV, cirrhosis	4.2	G2	ROH	ROH	ROH	ROH	None*
HCC11	62/M	Haemochromatosis, fibrosis	10.5	G2	ROH	ROH	NI	NI	None
HCC12	48/M	HCV, cirrhosis	2.5	G3	NI	ROH	NI	ROH	None*
HCC13	82/M	No cirrhosis	7.0	G2	NI	NI	NI	ROH	None*
HCC14	69/F	HBV, fibrosis	8.5	G2	NI	ROH	NI	ROH	None
HCC15	71/M	No cirrhosis	1.6	G2	NI	ROH	NI	NI	None
HCC16	81/F	No cirrhosis	18.0	G1	ROH	NI	NI	NI	None*
HCC17	64/M	HBV, cirrhosis	2.1	G2	NI	NI	NI	ROH	None
HCC18	48/M	HCV, cirrhosis	13.0	G2	ROH	ROH	NI	NI	None
HCC19	56/M	Alcohol, no cirrhosis	2.1	G1	NI	ROH	ROH	NI	None
HCC20	49/M	HBV, cirrhosis	10.0	G2	ROH	ROH	ROH	ROH	None
HCC21	37/F	No cirrhosis	5.0	G2	ROH	ROH	NI	ROH	None
HCC22	46/M	HCV, cirrhosis	6.0	G2	NI	NI	NI	ROH	None
HCC23	70/M	HCV, cirrhosis	2.2	G1	NI	ROH	NI	ROH	None*
HCC24	67/M	cirrhosis	5.0	G2	NI	ROH	NI	ROH	None*
HCC25	65/F	Alcohol, cirrhosis	3.8	G2	NI	LOH	NI	LOH	None*
HCC27	36/M	HBV, alcohol, cirrhosis	8.0	G3	ROH	ROH	NI	NI	None
HCC28	63/M	Alcohol, cirrhosis	3.5	G2	NI	NI	NI	NI	None
HCC29	56/M	Alcohol, no cirrhosis	4.0	G2	NI	NI	NI	NI	None*
HCC30	65/F	No cirrhosis	11.0	G1	LOH	LOH	NI	LOH	None*
HCC31	51/M	HCV, cirrhosis	2.5	G2	NI	ROH	NI	ROH	None*
HCC33	41/F	Alagille syndrome, cirrhosis	6.5	G3	NI	NI	ROH	ROH	None*
HCC34	75/M	No cirrhosis	5.0	G2	NI	ROH	ROH	NI	None
HCC35	23/F	HBV, cirrhosis	18.0	G2	NI	LOH	LOH	NI	None*
HCC36	57/M	HBV, cirrhosis	6.5	G2	ROH	ROH	NI	ROH	None
HCC37	61/F	HCV, fibrosis	4.2	G2	NI	ROH	ROH	ROH	None
HCC38	58/M	HCV, cirrhosis	2.9	G2	ROH	ROH	NI	NI	None
HCC39	72/M	Cirrhosis	6.5	G2	ROH	NI	NI	NI	None
HCC40	56/M	Haemochromatosis, fibrosis	4.8	G2	ROH	NI	NI	NI	None
HCC41	54/M	HCV, fibrosis	4.0	G2	ROH	ROH	ROH	NI	None
HCC42	69/M	Cirrhosis	4.5	G2	ROH	ROH	ROH	ROH	None
HCC43	58/M	Alcohol, cirrhosis	7.5	G3	NI	NI	NI	ROH	None

*PCR products have also been sequenced.

Four microsatellite markers from the 1p34.1 region (D1S421, D1S2677, D1S451, and D1S2802), which are located centromeric and telomeric of the *PRDX1* gene, were used.

HBV, hepatitis B virus; HCV, hepatitis C virus; LOH, loss of heterozygosity; ROH, retention of heterozygosity.

drawn from the files of the department of pathology, University Hospital Zürich, Switzerland, covering a period of 11 years (1992 to 2003). Tumour and non-tumorous liver tissue was macrodissected from 10 µm sections and DNA was extracted as described previously.¹⁵

LOH analysis

LOH was investigated using four polymorphic markers of the 1p34.1 region located within 0.8 Mb of the *PRDX1* locus (telomeric: D1S421; centromeric: D1S2677, D1S451, and D1S2802). Microsatellite markers were amplified from DNA extracted from HCC and corresponding non-tumorous liver tissue. Standard polymerase chain reaction (PCR) was performed using conditions and fluorescent labelled primers as obtained from the Ensembl Genome Browser (www.ensembl.org). PCR products were analysed on an automated DNA sequencer (model 373A; Applied Biosystems, Foster City, California, USA) using Gene Scan software (Applied Biosystems). Cases were classified as informative when two distinct alleles of similar intensity were found in the normal DNA. LOH was defined as present when an allele peak signal from tumour DNA was reduced by $\geq 50\%$ compared with the corresponding non-tumorous liver DNA.

PCR, denaturing gradient gel electrophoresis, and DNA sequencing

Primers amplifying the translated exons 2–6 were designed based on the reported genomic sequence of the human

PRDX1 gene (ENSG00000117450; www.ensembl.org): exon 2F, 5'-GTA GGT GAA GGC TGC TGG TT-3'; exon 2R, 5'-CAT CTC AAT GAG CCC AGT GTT-3'; exon 3F, 5'-TGC TAA CCA TGA CTC CGA TTT-3'; exon 3R, 5'-TTC ACA TGC CAA ATA GAC CAA-3'; exon 4F, 5'-GAA AGG GGA AAG AGG AAT GC-3'; exon 4R, 5'-GGC TTT CAG CCA ACT GGA TA-3'; exon 5F, 5'-AAC CTG TAT TGC TTT TCC TTT CA-3'; exon 5R, 5'-CAC CCC TTC ATA CCA CCA CT-3'; exon 6F, 5'-ATG GTG GTG CAT ACC ATT GA-3'; and exon 6R, 5'-GCA GCC TTG CAC TAA AAC AG-3'. PCR amplification, denaturing gradient gel electrophoresis, and cycle sequencing of PCR products were performed as described previously.¹⁵ Sequences were compared with the reported genomic sequence of the *PRDX1* gene (ENSG00000117450; www.ensembl.org).

RESULTS AND DISCUSSION

Three lines of evidence encouraged us to investigate genetic alterations of the *PRDX1* gene in human HCC. First, the *PRDX1* gene maps to the distal region of chromosome 1 (1p34.1), a region of frequent LOH in HCC. So far, three candidate tumour suppressor genes at the 1p36 region, the *PRDM2* (*RIZ*), *RUNX3*, and *TP73* genes, have been studied in HCC and no mutations have been detected, leaving the putative tumour suppressor gene(s) in this region unidentified.^{8–10} Second, HCCs show evidence of oxidative damage, suggesting that defects in ROS scavenging systems may contribute to the initiation and/or promotion of HCC.^{4–5} Because the *PRDX1* protein participates in the antioxidant

Take home messages

- The peroxiredoxin 1 gene (*PRDX1*) is a good candidate for the tumour suppressor gene implicated in the pathogenesis of hepatocellular carcinoma (HCC)
- However, loss of heterozygosity of four markers within 0.8 Mb of the *PRDX1* gene was seen in only three of 34 informative HCCs and no mutations or polymorphisms in the translated exons 2–6 of the *PRDX1* gene were found
- These results suggest that genetic alterations of the *PRDX1* locus are rare events in human HCC, indicating that other genes on chromosome 1p contribute to liver carcinogenesis

defence of hepatocytes, PRDX1 inactivation may enhance oxidative stress and thereby contribute to hepatocarcinogenesis. Third, HCCs occur with high penetrance in *Prdx1* mutant mice, suggesting a tumour suppressor function for PRDX1 in hepatocyte progenitor cells.¹⁴

“We cannot exclude the possibility that *PRDX1* inactivation by epigenetic mechanisms, such as promoter hypermethylation, may contribute to hepatocarcinogenesis”

To determine whether genetic alterations of the *PRDX1* gene are involved in human hepatocarcinogenesis, we first analysed this series of HCCs for LOH of four polymorphic microsatellite markers that are located telomeric and centromeric of the *PRDX1* locus on chromosome 1p34. Thirty four of 36 HCCs were informative for at least one of the four markers. Allelic loss of at least one microsatellite marker was seen in three of the 34 informative HCCs. Allelic loss of D1S421, D1S2677, D1S451, and D1S2802 was found in one of 14, two of 21, one of 10, and three of 26 informative cases, respectively (fig 1; table 1).

Our results agree with previous studies that also reported LOH at 1p34 in HCC, although with higher frequency (29%,¹⁶ 48–71%,¹⁷ and 15%¹⁸). These discrepancies may result from the use of different microsatellite markers and may indicate that LOH on chromosome 1p occurs distally of 1p34. Mutation analysis by PCR/denaturing gradient gel electrophoresis of the translated exons 2–6 of the *PRDX1* gene did not reveal band shift variants in the 36 HCCs. Subsequent DNA sequencing of exons 2–6 of 15 HCCs, including those with LOH at chromosome 1p34.1, revealed wild-type sequences.

We conclude that genetic alterations of the *PRDX1* gene are rare in HCC and that *PRDX1* is unlikely to be the target tumour suppressor gene of LOH on 1p. However, we cannot exclude the possibility that *PRDX1* inactivation by epigenetic mechanisms, such as promoter hypermethylation, may contribute to hepatocarcinogenesis.

ACKNOWLEDGEMENTS

We thank H Moch for critical reading of the manuscript. This work was partly supported by the Gebert R uf Foundation.

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Accepted for publication 23 March 2005

REFERENCES

- 1 Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. *Nat Rev Cancer* 2003;**3**:276–85.
- 2 Shimoda R, Nagashima M, Sakamoto M, *et al*. Increased formation of oxidative DNA damage, 8-hydroxydeoxyguanosine, in human livers with chronic hepatitis. *Cancer Res* 1994;**54**:3171–72.
- 3 Parola M, Robino G. Oxidative stress-related molecules and liver fibrosis. *J Hepatol* 2001;**35**:297–306.
- 4 Seki S, Kitada T, Sakaguchi H, *et al*. Pathological significance of oxidative cellular damage in human alcoholic liver disease. *Histopathology* 2003;**42**:365–71.
- 5 Ichiba M, Maeta Y, Mukoyama T, *et al*. Expression of 8-hydroxy-2'-deoxyguanosine in chronic liver disease and hepatocellular carcinoma. *Liver Int* 2003;**23**:338–45.
- 6 Suriawinata A, Xu R. An update on the molecular genetics of hepatocellular carcinoma. *Semin Liver Dis* 2004;**24**:77–88.
- 7 Nishimura T, Nishida N, Itoh T, *et al*. Discrete breakpoint mapping and shortest region of overlap of chromosome arm 1q gain and 1p loss in human hepatocellular carcinoma detected by semiquantitative microsatellite analysis. *Genes Chromosomes Cancer* 2005;**42**:34–43.
- 8 Fang W, Piao Z, Simon D, *et al*. Mapping of a minimal deleted region in human hepatocellular carcinoma to 1p36.13–p36.23 and mutational analysis of the RIZ (PRDM2) gene localized to the region. *Genes Chromosomes Cancer* 2000;**28**:269–75.
- 9 Mihara M, Nimura Y, Ichimiya S, *et al*. Absence of mutation of the p73 gene localized at chromosome 1p36.3 in hepatocellular carcinoma. *Br J Cancer* 1999;**79**:164–7.
- 10 Xiao WH, Liu WW. Hemizygous deletion and hypermethylation of RUNX3 gene in hepatocellular carcinoma. *World J Gastroenterol* 2004;**10**:376–80.
- 11 Wood ZA, Schroder E, Robin Harris J, *et al*. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem Sci* 2003;**28**:32–40.
- 12 Lee TH, Yu SL, Kim SU, *et al*. Characterization of mouse peroxiredoxin 1 genomic DNA and its expression. *Gene* 1999;**239**:243–50.
- 13 Immenschuh S, Baumgart-Vogt E, Tan M, *et al*. Differential cellular and subcellular localization of heme-binding protein 23/peroxiredoxin 1 and heme oxygenase-1 in rat liver. *J Histochem Cytochem* 2003;**51**:1621–31.
- 14 Neumann CA, Krause DS, Carman CV, *et al*. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature* 2003;**424**:561–5.
- 15 Mihic-Probst D, Perren A, Schmid S, *et al*. Absence of BRAF gene mutations differentiates Spitz nevi from malignant melanoma. *Anticancer Res* 2004;**24**:2415–18.
- 16 Sun M, Eshleman JR, Ferrell LD, *et al*. An early lesion in hepatic carcinogenesis: loss of heterozygosity in human cirrhotic livers and dysplastic nodules at the 1p36–p34 region. *Hepatology* 2001;**33**:1415–24.
- 17 Leung TH, Wong N, Lai PB, *et al*. Identification of four distinct regions of allelic imbalances on chromosome 1 by the combined comparative genomic hybridization and microsatellite analysis on hepatocellular carcinoma. *Mod Pathol* 2002;**15**:1213–20.
- 18 Koshikawa K, Nomoto S, Yamashita K, *et al*. Allelic imbalance at 1p36 in the pathogenesis of human hepatocellular carcinoma. *Hepatogastroenterology* 2004;**51**:186–91.