Rare allelic imbalances, but no mutations of the \textit{PRDX1} gene in human hepatocellular carcinomas

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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 (\textit{PRDX1}), an antioxidant enzyme, has been mapped to 1p34.1. Mice lacking \textit{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 \textit{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 \textit{PRDX1} gene was seen in three of 34 informative HCCs, but no mutations or polymorphisms in the translated exons 2–6 of the \textit{PRDX1} gene were found. These results suggest that genetic alterations of the \textit{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.\textsuperscript{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.\textsuperscript{2,3} Furthermore, hepatocellular carcinoma (HCC) shows evidence of oxidative DNA damage, as demonstrated by the formation of 8-hydroxy-2′-deoxyguanosine.\textsuperscript{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.\textsuperscript{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.\textsuperscript{7,8} So far, three candidate tumour suppressor genes on the 1p36 region, the \textit{PRDM2} (\textit{RIZ}), \textit{RUNX3}, and \textit{TP73} genes, have been studied in HCC, but no mutations have been detected.\textsuperscript{9–10}

Peroxiredoxin 1 (\textit{PRDX1})—also designated MSP23, OSF-3, or PAG—is an intracellular antioxidant protein with thioredoxin dependent peroxidase activity, which protects cells against oxidative stress by inactivating ROS, and is encoded by a single gene on chromosome 1p34.1.\textsuperscript{11} \textit{PRDX1} is highly expressed in the liver and was localised to hepatocytes and Kupffer cells in the rat.\textsuperscript{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.\textsuperscript{14} However, \textit{PRDX1} gene mutations have not been identified in human tumours.

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

\textbf{MATERIALS AND METHODS}

\textbf{Tumours, DNA extraction}

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

\textbf{Abbreviations:} LOH, loss of heterozygosity; PCR, polymerase chain reaction; PRDX1, peroxiredoxin 1; ROS, reactive oxygen species
The 1p34.1 region located within 0.8 Mb of the PRDX1 locus (telomeric: D1S421; centromeric: D1S2677, D1S451, and D1S2802), which are located centromeric and telomeric of the PRDX1 gene, were used. Four microsatellite markers from the 1p34.1 region (D1S421, D1S2677, D1S451, and D1S2802), which are located centromeric and telomeric of the PRDX1 gene (ENSG00000117450; www.ensembl.org), were amplified from DNA extracted as described previously. 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 on HCC. None of the 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. Second, HCCs show evidence of oxidative damage, suggesting that defects in ROS scavenging systems may contribute to the initiation and/or promotion of HCC. Because the PRDX1 protein participates in the antioxidant...
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.

“‘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%). 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.

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