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Mutation and methylation analysis of TP53 in adrenal carcinogenesis

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Abstract *Aim*: To investigate the role of coding region mutation and promoter hypermethylation of TP53 in adrenocortical cancer formation.
Methods: Twenty sporadic adrenocortical cancers (ACCs) and five normal adrenal tissue samples were available for analysis. Coding region mutation of TP53 in 20 ACCs was examined by polymerase chain amplification using intronic primers for exons 2–11 and direct sequencing of the product. In 10 ACCs and five normal adrenal tissue specimens, methylation of the 16 CpG sites within the TP53 promoter was examined using bisulphite methylation sequencing.
Results: Coding region mutation in TP53 was demonstrated in 5 of 20 ACCs. There were four mis-sense mutations and one frameshift mutation. Four of 5 patients with a TP53 mutation had metastases at diagnosis or detected soon thereafter and 3 of 4 died of disease within 12 months of surgical resection. No methylation was seen in the TP53 promoter in 10 ACC and the five normal adrenal tissues examined.
Conclusion: Coding region mutation in TP53 occurs in 25% of ACCs with a trend toward a poorer prognosis. Promoter methylation of TP53 is not present in ACC as a

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mechanism for tumour suppressor gene (TSG) inactivation and, therefore, other genes in the 17p13 region are implicated in adrenal carcinogenesis.

Introduction

ACC and 17p13

Cancer is the end result of the clonal expansion of a population of cells, which have acquired a number of non-lethal genetic alterations, principally involving oncogenes and TSG. According to Knudson's 2-hit hypothesis[1] both alleles of a tumour suppressor gene, if not imprinted, need to be inactivated for tumourigenesis to proceed. TP53 is a TSG located at 17p13 and encodes for a 393 amino acid nuclear phosphoprotein responsible for cell cycle regulation in response to a number of genotoxic and non-genotoxic stressors.[2] More than half of all human cancers have a mutation in TP53. Loss of heterozygosity at the 17p locus[3,4] and loss of 17p in comparative genomic hybridisation studies of adrenocortical tumours,[5,6] have consistently demonstrated that this region is implicated in adrenocortical tumourigenesis and as such, that TP53 is a strong candidate gene. Further supporting evidence is that adrenocortical cancer (ACC) is a manifestation of Li-Fraumeni syndrome in which patients carry a germline TP53 mutation.

ACC and TP53

Early studies examining for mutations in exons 5–8 of TP53 by Oghaki[7] and Reincke[8] showed mutations in only 3 of 15 (20%) and 3 of 11 (27%) ACCs, respectively. More recent interest in the role of TP53 in adrenocortical tumour progression was sparked by a report by Ribeiro[9] demonstrating a germline R337H mutation in exon 10 of TP53 in 35 of 36 (97%) children from southern Brazil who had apparently sporadic ACC. This finding was verified by Latronico[10] who also examined the tumours and germlines of 37 adults from Brazil with adrenocortical tumours and discovered that 5 of 37 (13.5%) of the tumours showed the R337H mutation in exon 10. It is now acknowledged that the R337H mutation is a signature low penetrance allele causing an exclusive pre-disposition to ACC.[11] Most recently, a group from Italy has published a 70% mutation rate in 10 ACCs examined for mutation in TP53.[12]

DNA methylation

DNA methylation of specific sites within the promoter region of a TSG is an alternative mechanism for inactivation of a TSG. This has been seen in genes such as p16 (cell cycle control), p15 (cell cycle control), MLH1 (mismatch repair), THBS1 (angiogenesis inhibition), TIMP-3 (metastasis inhibition) and ER (growth suppression) in a variety of cancers including the oesophagus, colon, prostrate, pancreas, lung, bladder and blood.[13–15] Interestingly, although TP53 is the most commonly mutated gene in human cancer, little work has been performed on examining methylation in the promoter region of this gene. Recent work has demonstrated that promoter methylation in TP53 can lead to decreased expression of the gene.[16]

Against this background, we undertook to examine the role of TP53 in adrenocortical tumourigenesis by examining for coding region mutations in exons 2–11 of the gene in 20 ACCs and examining for the presence or absence of methylation in the TP53 promoter in 10 ACC and five normal adrenal tissues.

Methods

Patients and tumours

Ethics approval for the study was obtained from the Northern Sydney Area Health Service Ethics Committee and Ethics Committees of participating institutions. The study was performed in accordance with the ethical standards of the Helsinki declaration of 1975. Tissue samples from 20 patients with sporadic, histologically proven adrenocortical tumours were available for analysis. Patients with adrenal tumours as part of a known familial syndrome were excluded on clinical grounds. There were six males and 14 females with a mean age of 51 years. Patient data is summarised in Table 1. Tumour tissue was obtained at surgery from the central part of the lesion and snap frozen at −80 °C in liquid nitrogen. DNA extraction was performed from fresh frozen tissue