Analysis of the insertion/deletion polymorphism of the human angiotensin converting enzyme (ACE) gene in patients with renal cancer

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Summary The angiotensin I-converting enzyme (ACE) contains an insertion/deletion (I/D) polymorphism, with the DD genotype associated with benign renal diseases. The distribution frequencies of the D and I alleles, and the DD, DI and II genotypes were determined in DNA extracted from kidney tissues of 58 renal cancer patients. The observed frequencies in patients who develop renal cancer was not significantly different than the normal population. © 2000 Cancer Research Campaign

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The angiotensin I-converting enzyme (ACE) is found in the plasma membrane of vascular endothelial cells and on a variety of extravascular tissues, while the soluble form of ACE circulates in plasma. The inter-individual variability of human plasma and cellular ACE levels is predominantly under the influence of a genetic polymorphism in the *ACE* gene (Alhenc-Gelas et al, 1997), which corresponds to the presence or absence of a 287-bp Alu-type sequence located in intron 16. Individuals homozygous for the insertion (I genotype) have lower plasma and membrane-bound ACE levels than those homozygotes without the insertion (deletion, or D genotype), while heterozygotes display intermediate levels (DI genotype) (Rigat et al, 1990). The DD genotype is significantly associated with diabetic nephropathy in type I diabetes (Marre et al, 1994), progressive renal dysfunction leading to chronic renal failure in IgA nephropathy (Yoshida et al, 1995), renal artery stenosis (Missouris et al, 1996) and end-stage renal failure in polycystic kidney disease (Baboolal et al, 1997). We considered whether the DD genotype occurs with an increased frequency in patients developing renal cell carcinoma. We therefore analysed DNA derived from normal kidney tissue of 58 patients who underwent nephrectomy for primary renal tumours for the prevalence of the I and D alleles, and for the incidence of the DD genotype.

MATERIALS AND METHODS

Tissue specimens and PCR analysis

Surgically removed renal tumour specimens were obtained from 58 random patients undergoing radical nephrectomy at Memorial Sloan-Kettering Cancer Center (MSKCC) and processed as

described (Nanus et al, 1990). Polymerase chain reaction (PCR) was performed on DNA derived from the tumours using gene-specific oligonucleotide primers as previously reported (Albino et al, 1991; Nanus et al, 1993a). A single PCR product of 190 bp product was genotyped DD, of 490 bp product was genotyped II, and if both 190 bp and 490 bp were present the sample was genotyped DI (Rigat et al, 1990). Any DNA sample genotyped DD underwent a second amplification with a nested primer to confirm the genotype (Shanmugan et al, 1993). Amplification of the *ACE* gene used the following amplimers: sense 5'-CTGGAGACCACTCCCATCCTTTCT-3' and anti-sense 5'-GATGTGCCATCACATTCGTCAGAT-3' (Rigat et al, 1992). The DD genotypes were confirmed using a nested sense primer 5'-TTTGAGACGGAGTCTCGCTC-3' as described (Shanmugan et al, 1993). A χ^2 goodness of fit test was performed to determine if the observed proportion match with the control proportion (Armitage and Berry, 1987). Two-sided *P*-values are reported.

RESULTS

DNA was extracted from normal kidney obtained at nephrectomy from 58 patients undergoing surgical resection of primary renal tumours. There were of 36 males and 22 females with a median age of 64 (range 32–83). Histological review revealed 46 clear cell carcinomas and 12 non-clear cell histologies (four papillary carcinomas, four chromophobe carcinomas, four oncocytomas). DNA was amplified using gene-specific primers for intron 16. Representative PCR results are illustrated in Figure 1. The frequency of the ACE D and I alleles for all 58 patients was 0.612 and 0.388 respectively. This frequency was similar to that observed in a control population (Table 1). The observed frequencies of 0.362, 0.50 and 0.138 for the DD, DI and II genotypes respectively were not significantly different from the frequencies predicted by Hardy–Weinberg equilibrium ($\chi^2 = 2.01$; *P* = 0.4). Subset analysis of the distribution of the D and I alleles in clear-cell versus non-clear cell histology revealed a 75% incidence of the D allele in patients with non-clear cell renal tumours ($\chi^2 = 3.7$, *P* = 0.06), however, there were only 12 samples in this cohort.

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Figure 1 PCR amplification of ACE alleles. Representative data of DNA extracted from normal kidney tissue amplified using insertion-specific primers for intron 16, and the PCR product separated on an agarose gel. Representative samples shown: I genotype (lane 1); DI genotype (lanes 2 and 5); and DD genotype (lanes 3 and 4). MW = molecular weight marker

Table 1 Distribution of ACE alleles and genotypes in DNA of patients with kidney cancer

	CC ^a (n = 46)	Non-CC (n = 12)	All (n = 58)	Controls ^c (n = 553)
ACE alleles				
D	53 (0.576) ^b	18 (0.75)	71 (0.612)	0.555
I	39 (0.424)	6 (0.25)	45 (0.388)	0.445
ACE genotypes				
DD	15 (0.341)	6 (0.5)	21 (0.362)	0.284
DI	23 (0.522)	6 (0.5)	29 (0.5)	0.533
II	8 (0.182)	0	8 (0.138)	0.183

^aCC = clear cell carcinoma; Non-CC = non-clear cell. ^bNumber (frequency).

^cOther population control as described (Schachter et al, 1994).

DISCUSSION

The distribution of the ACE D and I alleles and the ACE genotypes in 58 patients diagnosed with renal cell carcinoma was similar to that observed in the normal population, and did not associate with the development of renal cancer. These data contrast multiple studies in which the DD polymorphism, which results in elevated plasma and membrane bound ACE, is associated with the development of benign renal diseases (Costeroousse et al, 1997). Elevated ACE levels lead to increased angiotensin II production and bradykinin inactivation, and it is the increase in angiotensin II that is believed to contribute to the numerous pathological states associated with the DD genotype. Recent data indicate that angiotensin II functions at the cellular level as a growth factor (Wolf and Neilson, 1993; Wolf, 1995) and is essential for normal growth and development of renal tubules (Alcorn and Maric, 1996).

Despite that the current study which did not identify an increased frequency of the DD polymorphism does not implicate elevated ACE and consequently increased angiotensin II levels in the development of renal tumours, a number of recent studies do suggest that the renin-angiotensin system plays a role in renal carcinogenesis. Angiotensin II can induce the synthesis of basic fibroblast growth factor (bFGF) (Itoh et al, 1993) which has been implicated in the development and progression of renal cancers (Nanus et al, 1993b). The ACE inhibitor Captopril significantly reduces the tumour size of renal cancer xenografts in athymic mice in a dose-dependent fashion (Hii et al, 1998). We have shown that overexpression of aminopeptidase A, which converts angiotensin II to angiotensin III, in renal cancer cells inhibits anchorage-independent growth and tumorigenicity (unpublished data).

Finally, one recent study suggests that long-term use of ACE inhibitors may protect against cancer (Lever et al, 1998). Taken together, these data begin to implicate ACE and its substrates including angiotensin II in the pathogenesis of renal cancers, and indicate that the involvement of the tissue renin-angiotensin system in this disease warrants further study.

In summary, the distribution of the ACE polymorphism in 58 patients with renal cell carcinomas is not significantly different than the expected incidence in patients without renal cancers. These data suggest that elevated ACE levels do not predispose to the development of renal tumours. Further studies are needed to define the involvement of the renin-angiotensin system in renal carcinogenesis.

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