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Multifocal renal cell carcinoma: a cytogenetic study

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Summary. A case of multifocal renal cell carcinoma (RCC) is reported. A detailed cytogenetic study revealed diverse clonal chromosomal aberrations in both tumours and demonstrated the multiple origin of these tumours. The tool of chromosome analysis in discriminating between multifocal renal cell tumours and local metastasis, as well as in the determination of renal adenomas and carcinomas is discussed.

Key words: Renal cell carcinoma – Multifocal tumour development – Chromosome aberrations

Introduction

Renal cell carcinomas (RCC) are mainly solitary lesions occurring with equal frequency in both kidneys. Synchronous or asynchronous bilateral RCC's and the multifocal development of RCC's, are relatively rare in the general population but they occur with higher frequency in some genetic syndromes. Bilateral RCC's are associated with von Hippel-Lindau disease, which predisposes to the development of renal cancer (Richards et al. 1973). In familial RCC with a constitutional chromosomal defect (Cohen et al. 1979) or normal constitutional karyotype (Pathak et al. 1982), both bilateral and multifocal occurrence of RCC was observed, with significantly higher incidence than that found in the unrelated population (Vermillion et al. 1972).

Multiple RCC's occurring in the same kidney have been observed in 2–4% of the cases without hereditary diseases (Schubert 1984). Histological analysis cannot answer the question whether such lesions arise due to independent primary origin of separate tumour, or due to spread from a single primary tumour. A number of RCC's are histologically heterogeneous, and the metastases sometimes differ in their histological pattern from the primary tumour. Therefore, no absolute histological parameter exists to distinguish between true multifocal RCC's and local metastasis in a given kidney. Recently, the technique of short term culture and detailed chromosome analysis of solid tumour has become available, and this has opened up definitive analysis for clonality of such tumours. We report here a case with two RCC's in the same kidney, analysed using short-term cultures and banded metaphases to prove the simultaneous occurrence of two distinct tumours.

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Case report

A 73-year-old female was admitted to the hospital for cerebral apoplexy. By examination with ultrasound and subsequent computerised tomography, a tumour mass in the right kidney was found. Right nephrectomy was performed, with adrenalectomy and regional lymphadenectomy. The nephrectomy specimen weighed 350 g and measured 15 × 6 × 3,5 cm. In the lower pole of the kidney a well-encapsulated, yellow tumour of 5,8 cms diameter was found. In the middle part of this kidney another, sharply demarcated, small subcapsular tumour of 7 mms diameter was detected.

Histologically, the larger tumour (designated tumour I) was composed of clear cells arranged trabecularly and showing uniform small, partly picnotic nuclei (Fig. 1). The central tumour areas were fibrotic, showing remnants of small tumour cell nests. In several slides observed, no mitotic activity was found. Focal infiltration of the fibrotic pseudocapsule was observed but no venous or renal pelvis infiltration was found. The regional lymph nodes were free of tumour metastasis.

The small subcapsular tumour (designated tumour II) was encased by a thick fibrous capsule (Fig. 2) and showed mainly papillary, but in some areas tubulo-papillary structures. The papillae were lined by a mostly single row of cells showing cytoplasmic features identical to those of the classical granular cell renal carcinoma. The cytoplasmic granules were PAS-positive and diastase-labile. This tumour showed a slight degree of nuclear pleomorphism and hyperchromatic nuclei in a small area, with occasional mitotic cells (Fig. 2, inset). The vascular-

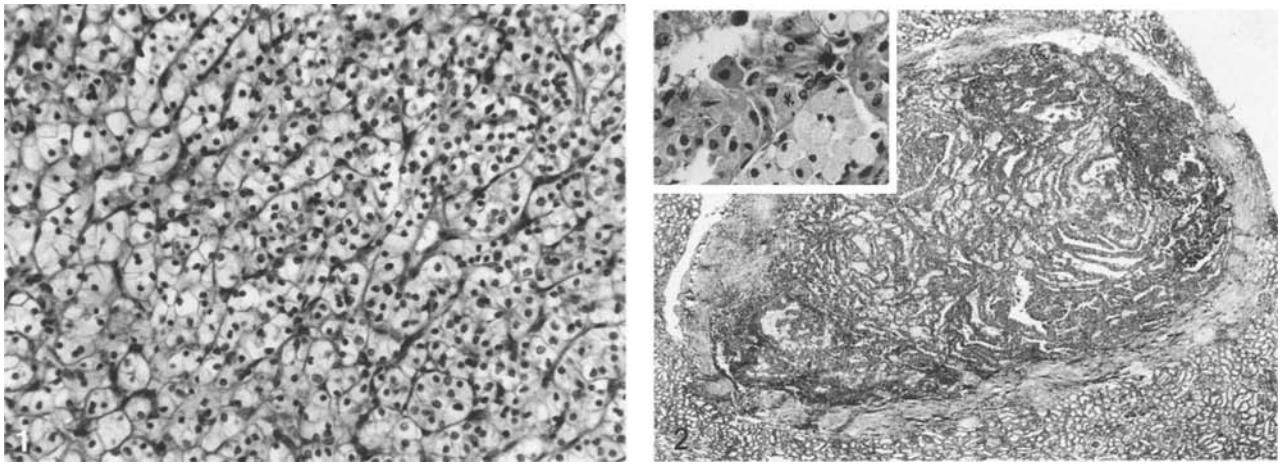

Fig. 1. Histological picture of tumour I showing trabecularly rearranged clear cells. (H & E, ×200)

Fig. 2. Low power microphotograph of the small subcapcular tumour composed of tubulo-papillary structures of granular cells (H & E, ×15). *Insert*: Higher magnification of the same tumour showing a mitotic tumour cell (H & E, ×280)

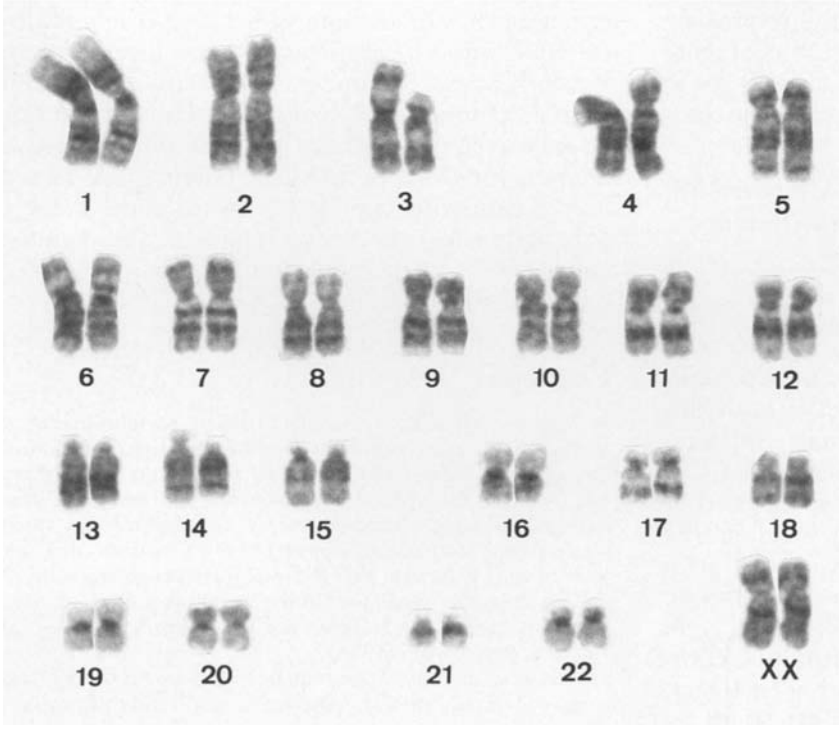

Fig. 3. G-banded karyotype of a stemline cell of tumour I: 46,XX,del(3) (p13)

ized stalks of the papillary structures were infiltrated by numerous macrophages displaying an abundant granular cytoplasm. There was a great deal of fibrosis and chronic inflammation at the tumour-parenchyma border, with infiltration by lymphocytes, plasma cells and haemosiderin-loaded macrophages. Some arteries feeding the tumour were narrowed by subendothelial sclerosis.

Materials and methods

A small area free of fibrosis was excised from tumour tissue for the preparation. One portion of the tumour sample was fixed in formaldehyde and used for histological examination as "reference slide" for tissue cultures. From the larger tumour two samples (IA and IB) were excised separately. Specimens of tumour tissue were cut into appr. 2–3 mm^3 pieces and washed twice in medium. The small tissue fragments were then incubated in 0,1% collagenase (Worthington CLS III) dissolved in culture medium (RPMI 1640 supplemented with 15% fetal calf serum and antibiotics) for 60 min at 37° C. Then the tissue fragments were washed twice in medium, dispersed vigorously with a Pasteur pipette and the small cell clusters were seeded in culture.

Chromosome analysis was carried out on the third and fifth days of primary cultures by adding 0,1 μg/ml colchicine for 60 min. The cells were then treated with 0,075 mol potassium chloride solution for 20 min and fixed with methanol-acetic

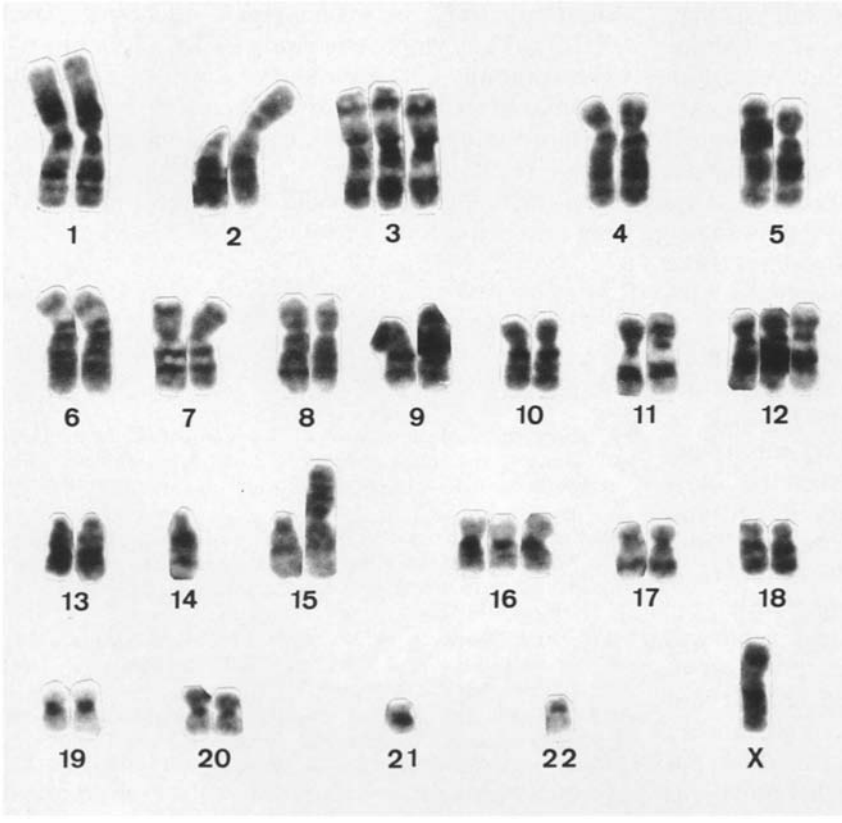

Fig. 4. A representative G-banded karyotype of a tumour cell from tumour II: 45,X,−X, del(2)(p11),+3,+12,−14, der(15)*t*(2;15)(p11;p11),+16,−21,−22

acid (3:1) three times. Chromosome preparations were made by air drying technique. Karyotype analysis was performed using GTG- and CBG-techniques. Each well-banded metaphasis was karyotyped according to the ISCN (1985).

From tumour I two samples, excised separately, were karyotyped. From the first probe IA a total of 41 metaphases was analysed with G-banding. The modal chromosome number was 46, and in 32 cells a deletion of the short arm of chromosome 3 was the only karyotypic change (Fig. 3). In two cells additional loss of chromosome 14, and in a single cell additional changes in form of translocation 1;20 and deletion 14q were found. The remaining 8 cells show the stemline karyotype with chromosome losses of nonclonal character. In the second sample I B 24 G-banded metaphases were analysed. In 11 cells, similarly to the first probe, a deletion 3p was found as a sole karyotype change. The other 13 metaphases, however, were characterized by an additional clonal rearrangement in the form of reciprocal translocation between chromosomes 6 and 12: 46,XX,del(3)(p13),rcp(6;12)(p11;q13). Both reference slides corresponding to samples I A and I B showed precisely the same histological picture, a well-differentiated clear cell carcinoma without any nuclear polymorphism or mitotic activity.

From tumour II a total of 23 G-banded metaphases was karyotyped. This tumour showed a modal chromosome number of 45. All but one cells were characterized by a translocation between chromosomes 2 and 15. In 16 cells identical chromosome changes were found (Fig. 4). Four other cells show this stemline karyotype, each with an additional change, *t*(1;6), *t*(1;3), *t*(1;3) or *t*(1;19). In a single cell with 55 chromosomes only numerical chromosome changes were found. For this tumour the routine histological preparation served as the reference slide.

Discussion

In the case described here, two histologically different renal tumours were observed in a nephrectomy specimen and chromosome analysis revealed clonal karyotype changes in both. Two questions arise in relation to this case; are multiple tumours occurring in the same kidney as distinct tumours, or does one represent a metastasis from the other? Are renal tumours smaller than 1 cm in diameter always renal adenomas or do they represent small RCC's as well as true adenomas?

If the two tumours occurring in the same kidney show different histological and cytological picture, multifocal tumour development can be suggested. However, a significant number of primary RCC's are histologically heterogeneous and different growth patterns and cell morphology are seen in different tumour areas. Furthermore, the metastases of RCC may differ markedly, both in histological pattern and in degree of differentiation, from the primary tumour. Therefore, a multifocal development of RCC would often defy definition by histological examination alone. Renal carcinomas are characterized by marked karyotype changes (Kovacs et al. 1987), with at least one structural chromosome aberration marking all cells of a

given tumour. Therefore, cytogenetic analysis may be helpful in distinguishing between cases with local metastases in the same kidney and cases with multiple tumour formation. In the present case both tumours show well-defined karyological changes, and none of the detected chromosome aberrations was common to both tumours. One may therefore conclude that these tumours are not descended from the same progenitor cell, and the multicentric development of two independent tumours in the same kidney was involved.

Tumour II was smaller than 1 cm (7 mm in diameter). Solely on the basis of the histological picture of this small tumour, it is very difficult to determine whether it is a small papillary carcinoma or a small adenoma. It is well-established that most tumours develop from a normal diploid cell and that malignant tumours acquire more and more secondary chromosome aberrations during their clonal progression (Nowell 1976). Recently, the specificity of chromosome 3 rearrangement was suggested to be instrumental in the initiation of RCC's (Cohen et al. 1979; Yoshida et al. 1986; Kovacs et al. 1987). Unfortunately, there are no reports on cytogenetical analysis of renal adenomas. We may speculate that true renal adenomas consist of normal diploid cells, or show minimal chromosomal changes, perhaps varying from those found in RCC's. In considering the existing data concerning the cytogenetics of renal tumours, the small tumour in the present case cannot be evaluated as a renal adenoma. It showed clonal chromosomal aberrations, and we therefore felt justified in identifying it as a small papillary RCC.

The problem of renal adenomas versus small, slowly growing carcinomas has been discussed for many years by pathologists (Bennington and Beckwith 1975; Mostofi 1981). There are no morphological features of these tumours distinguishing them from each other (Fischer and Horva 1972). "Renal cell tumours of doubtful dignity", namely well-differentiated tumours between 1 and 3 cm in diameter, also lack more definitive determinants which enable us to evaluate the clinical prognosis (Thoenes et al. 1986). We suggest that chromosome analysis of small, histologically doubtful tumours may be helpful for the better understanding of the biology of these tumours and perhaps for discrimination between renal adenomas and RCC's. The cytogenetic study of RCC's is in progress but the chromosomal features of renal adenomas are as yet unknown. Therefore, in addition to morphological studies, chromosome analysis of small renal tumours of "doubtful dignity" and true renal adenomas would be valuable in providing a solution to this problem.

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