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Determination of telomerase activity for differential analysis of multifocal renal cell carcinomas

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Determination of telomerase activity for differential analysis of multifocal renal cell carcinomas. Secondary tumors are found in approximately 12 to 22% of all renal cell carcinoma, and their origin is currently unknown. To determine their potential for malignancy, we examined the telomerase activity of primary tumors and secondary lesions, and found that 86% of the lesions had an identical telomerase status as the related primary tumors, and thus probably share their malignancy potential.

Renal cell carcinoma (RCC) constitutes approximately 90% of all kidney tumors. Twelve to 20% of cases contain multifocal tumor areas that are detected after histopathological examination. Whether these secondary tumors are derived from the primary tumor or have an heterogeneous origin is not known. Also, the malignant potential of the secondary tumors and whether or not they characterize a higher risk for metastases are not yet clear. Therefore, a clearer differentiation of the nature of multifocality in RCCs is important especially with respect to a nephron-sparing surgery.

Recently, the determination of the activity of the enzyme telomerase has proved to have diagnostic and prognostic value in a wide range of tumors. As the maintenance of telomere length is elementary for indefinite cell proliferation, telomerase activity is directly linked to the potential immortal status of these cells. Furthermore, telomerase activity is closely associated with the malignancy of tumors, as enzyme activity is often present in malignant tumors but is not present in their benign counterparts.

We have previously shown that telomerase is active in approximately 93% of all tested primary RCCs independent of histopathological classification or grading and staging [1]. Thus, telomerase reactivation seems to be a critical and necessary step in the tumorigenesis of all kinds of primary RCCs. In order to characterize the RCCs exhibiting multifocal lesions in more detail, we analyzed telomerase status in secondary lesions and corresponding primary tumors from 20 different patients using a polymerase chain reaction (PCR)-based, highly sensitive nonradioactive detection method.

Key words: telomerase, tumor, metastases, cancer.

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METHODS

Samples

The study includes 20 patients exhibiting primary RCCs and at least one secondary lesion. All tumor probes were obtained by surgical resection. The tissue probes were snap frozen in liquid nitrogen immediately after resection and were stored at −80°C.

Histopathological examination

Primary tumors and secondary lesions were analyzed histologically according to the classification of Thoenes. Tumor stage was defined according to the TNM system.

Cell culture and chromosome analysis

All tissue samples were cultured as described previously by Junker et al [2]. Briefly, the minced and collagenase/trypsin treated specimens were resuspended in Amniomax 100 (GIBCO BRL, Grand Island, NY, USA) and incubated at 37°C and 5% CO_2. Cytogenetic analysis was carried out on cultured cells using standard cytogenetic techniques (GTG banding).

Telomerase assay

For the telomeric repeat amplification protocol (TRAP) assay, the S100 protein extract was isolated from 1 million cells of cultured secondary tumors or approximately 50 mg of the homogenized frozen tissue samples (all tested primary tumors and secondary lesions of cases 175, 186, 238, and 251) as described by Kim et al [3]. The concentration of protein was measured using the Bio-Rad protein assay kit (Bio-Rad, Richmond, CA, USA). An aliquot of the extract representing 1.5 μg of

protein solution or a 10 μm cryostat section (in cases 238, 321, 348, 361, 363, and primary and secondary tumor #1 from case 180) was mixed with 75 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 20 mM $(NH_4)2SO_4$, 0.1 mM phenylmethylsulfonyl fluoride, 50 mM dNTPs, 0.05 μg TS oligonucleotide (5′-AATCCGTCGAGCAGAGTT-3′, labeled with infrared dye IRD 800; Boehringer-Mannheim, Mannheim, Germany), and 0.5 μg T4 gene 32 protein (Pharmacia, Uppsala, Sweden) in a total volume of 25 μl. The reaction mixture was incubated for 10 minutes at 23°C for telomerase-mediated extension of the TS primer and was then heated at 94°C for 90 seconds for inactivating the telomerase. After adding 0.05 μg CX primer (5′-CCCTTACCCTTACCCTTACCCTTA-3′) and 1 unit of Taq DNA polymerase (Eurogentec, Seraing, Belgium), the probes were subjected to 31 PCR cycles of 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 45 seconds. For control experiments, protein extracts were RNAse pretreated to a final concentration of 0.05 mg/ml before performing the TRAP assay. Finally, 4 μl of each PCR product were separated by electrophoresis on a 6% denaturing polyacrylamide gel using a LI-COR DNA sequencer. The laser detection system of the LI-COR makes visible the extended and amplificated PCR products as an increasing DNA ladder.

RESULTS

There was no correlation of multifocality or telomerase activity with a specific cell type or grading and staging of the RCCs (Table 1). Nine out of 14 (64%) tested primary tumors displayed telomerase activity. In total, 24 different secondary lesions from 20 patients with primary RCC were analyzed for telomerase activity. Thirteen (54%) of these secondary lesions were telomerase positive (Fig. 1 and Table 1). With the exception of case 198, all telomerase-positive primary tumors exhibited at least one secondary lesion with active telomerase. Twelve out of 14 (86%) cases with primary and secondary tumor tested in parallel displayed identical telomerase status. Eleven secondary lesions derived from 10 different patients showed no telomerase activity. In four of these cases (151, 163, 321, and 361), the corresponding primary tumors were also telomerase negative. Only one of the primary tumors (case 363) tested to be telomerase negative showed a secondary tumor that was telomerase positive. In this case, the histopathological feature and the karyotype also were different between the primary and secondary tumors. Two cases (180 and 198) showed telomerase-positive primary tumors, whereas the corresponding secondary tumors were telomerase negative. Case 180 exhibited four different secondary lesions. Two of them displayed telomerase activity, whereas the other two lacked telomerase activity.

Table 1. Telomerase activity in primary and secondary tumors of renal cell carcinoma (RCC)

Case no/area	Histopathologic feature	Staging/ grading	Telomerase activity	Karyotype
118/pr. tumor	clear cell	pT2/G1	nt	1
sec. tumor		G1	−	
132/pr. tumor	clear cell	pT3b/G1	nt	3
sec. tumor		G2	+	
141/pr. tumor	clear cell	pT3a/G2	nt	3
sec. tumor	adenoma	G2	−	
151/pr. tumor	chromophobe	pT2/G2	−	1
sec. tumor		G2	−	
152/pr. tumor	clear cell	pT1/G1	+	1
/sec. tumor 1		G1	+	
sec. tumor 2			+	
159/pr. tumor	chromophobe	pT2/G2	nt	1
sec. tumor		G2	+	
163/pr. tumor	chromophobe	pT2/G2	−	2
sec. tumor	chromophobe	G2	−	
175/pr. tumor	clear cell	pT3a/G2	+	nt
sec. tumor			+	
180/pr. tumor	clear cell	nt	+	2
sec. tumor 1	clear cell		+	2
sec. tumor 2	clear cell		−	3
sec. tumor 3	clear cell		+	2
sec. tumor 4	clear cell		−	3
181/pr. tumor	clear cell	pT2	+	2
sec. tumor	clear cell		+	
185/pr. tumor	clear cell	pT2/G1	nt	3
sec. tumor		G1	−	
186/pr. tumor	clear cell	pT3a/G2	+	1
sec. tumor		G2	+	
198/pr. tumor	eosinophil	pT3a/G3	+	2
sec. tumor		G3	−	
202/pr. tumor	clear cell	G2	nt	3
sec. tumor	adenoma	G2	−	
238/pr. tumor	clear cell	pT3b/G2	+	nt
sec. tumor			+	
251/pr. tumor	nt	nt	+	nt
sec. tumor			+	
321/pr. tumor	clear cell	pT3a/G2	−	nt
sec. tumor	clear cell		−	
348/pr. tumor	clear cell	pT2/G3	+	nt
sec. tumor	clear cell		+	
361/pr. tumor	chromophil	pT2/G3	−	
sec. tumor	chromophil		−	nt
363/pr. tumor	chromophil	pT2/G2	−	3
sec. tumor	clear cell	G1	+	

Numbers signify: (1) concordant karyotypes of primary and secondary tumors; (2) concordant clonal chromosomal aberrations: (3) different karyotypes; nt, not tested. Abbreviations are: pr., primary; sec., secondary.

DISCUSSION

A comparison of biological parameters of secondary and respective primary tumors was carried out to more clearly define the nature of multifocality in RCCs. The identical telomerase status in primary and secondary lesions in 86% of analyzed cases indicates an clonal origin and analogous biological behavior of these secondary lesions. This hypothesis of clonal conformity of primary and secondary tumors is confirmed by the concordance of the cytogenetic and telomerase data, as all primary tumors and corresponding secondary lesions displaying identical telomerase status also have concordant chromosomal alterations or concordant karyotypes. In the

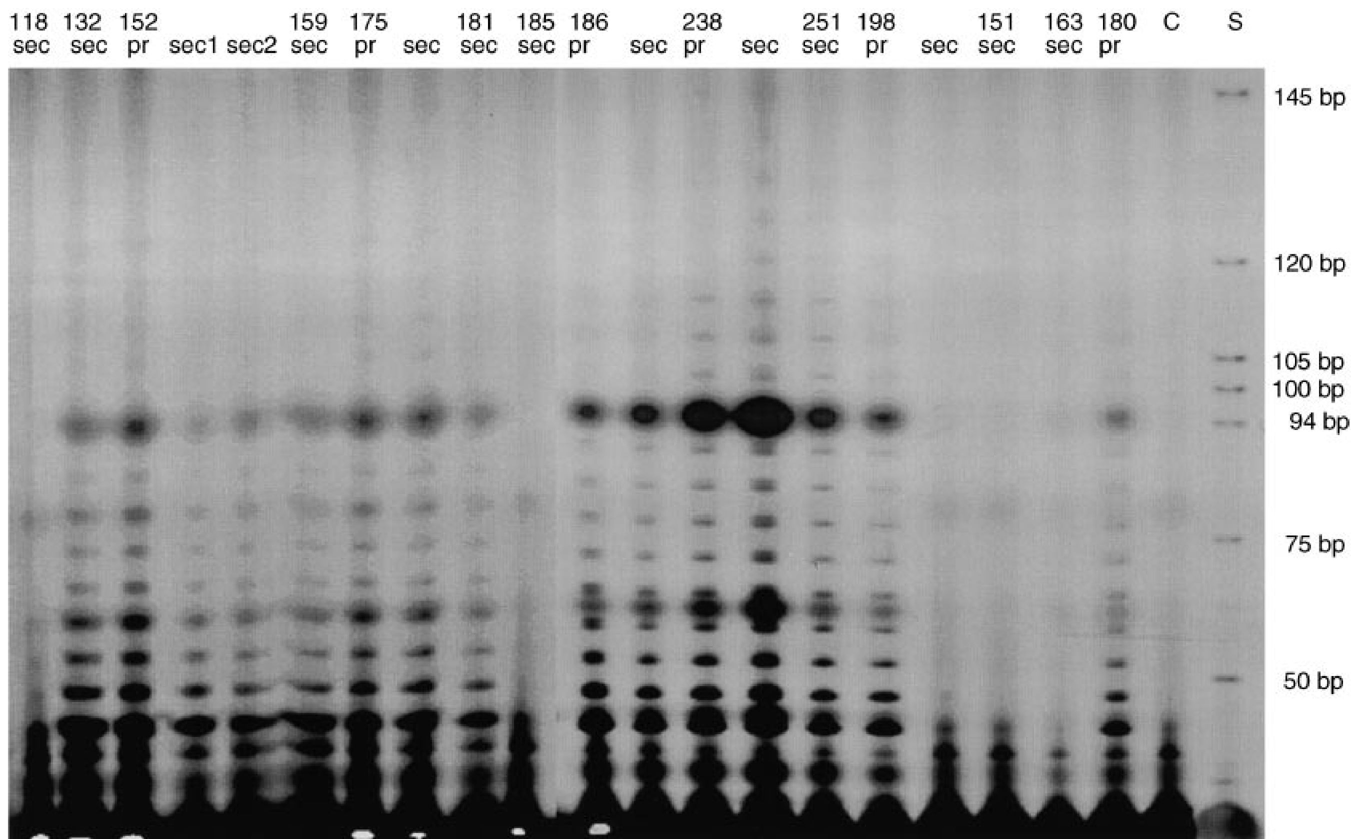

Fig. 1. Detection of telomerase activity in renal cell carcinoma (RCC). Lanes 1 through 20 are the results of the telomeric repeat amplification protocol (TRAP) assay from primary and secondary tumors. Active telomerase is visible as an increasing DNA ladder starting at 40 bp. Abbreviations are: C, control assay (TRAP with RNAse treated protein extract); S, molecular weight standard.

two cases (180 and 198) with a telomerase-negative secondary lesion but a telomerase-active primary tumor, the secondary tumors may have a different clonal origin. It is also possible that these results are the consequence of artifacts from analyzing cell cultures. Because of the usually small size of the secondary tumors, in most cases, cultured cells of the RCCs were used for the telomerase assay. This implies a risk of cultivating and analyzing contaminating normal cells, and may be true especially for tumors without telomerase activity and normal karyotypes (secondary tumors of cases 118, 141, 151, 180, 185, and 202). Thus, more secondary tumors with active telomerase may exist in reality. For a better assessment of the results, the analysis of cryostat sections of the tumor specimens (as it could be performed in cases 186 and 238 and in the secondary tumor #1 of case 180) would be worthwhile.

As a consequence of our data, it is obvious that most secondary lesions are more likely the result of intrarenal metastasis than the result of multifocal genesis. We conclude by the concordance of cytogenetic and enzymatic data that secondary tumors may have malignant potential similar to that of the related primary tumors. A higher malignant potential of telomerase-positive (primary and secondary) tumors may be proposed, as only telomerase-active primary tumor exhibited telomerase-active secondary tumors (with the exception of case 363), and most secondary tumors are telomerase positive. Also, the absence of telomerase activity in secondary tumors of cases 141 and 202 both classified as benign adenomas may underscore this presumption.

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REFERENCES

1. Fiedler W, Dahse R, Schlichter A, Junker K, Kosmehl H, Ernst G, Schubert J, Claussen U: Telomerase activity and telomere length in different areas of renal cell carcinoma. *Int J Oncol* 9:1227–1235, 1996
2. Junker K, Schlichter A, Junker U, Knofel B, Kosmehl H, Schubert J, Claussen U: Cytogenetic, histopathologic, and immunologic studies of multifocal renal cell carcinoma. *Cancer* 79:975–981, 1997
3. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW: Specific association of human telomerase activity with immortal cells and cancer. *Science* 266:2011–2015, 1994