Telomere Length in Peripheral Blood Predicts Survival in Clear Cell Renal Cell Carcinoma

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Abstract

Telomeres are repetitive structures located at chromosome ends. Previous studies have indicated that blood cell telomeres may serve as a biomarker for cancer risk. In addition, we recently reported that blood telomere length predicted survival in patients with breast cancer. In the present study, we examined whether blood telomere length may act as a predictor for survival in newly diagnosed patients with clear cell renal cell carcinoma. Furthermore, we analyzed telomere length in tumor samples and corresponding kidney cortex. Relative telomere length (RTL) was measured on extracted DNA using real-time PCR. Interestingly, and in line with our previous findings in breast cancer, patients with the longest blood telomeres (fourth quartile) had a significantly worse prognosis compared with patients with shorter blood RTL (P = 0.005). A highly significant association was found between long blood telomeres and a poor outcome in patients with nonmetastatic disease (P < 0.001), whereas patients with distant metastases had a poor survival regardless of blood RTL (P = 0.432). No correlations were found between blood RTL and various clinical variables, such as erythrocyte sedimentation rate, hemoglobin, and thrombocyte count. Multivariate Cox regression analysis verified long blood RTL as an independent negative prognostic marker. In contrast, telomere length in kidney cortex and tumor tissue did not predict survival. In conclusion, our results indicate that blood RTL may predict kidney cancer survival, with implications for future treatment strategies. [Cancer Res 2009;69(7):2896–901]

Introduction

Telomeres, the protective structures capping our chromosome ends, normally shorten with each cell division due to the end-replication problem (1). Because telomere erosion below a critical length triggers senescence, telomeres have been described as a molecular clock, regulating the replicative life span of eukaryotic cells (2). Critically short telomeres are associated with genetic instability and an increased cancer risk (3). Tumor cells often have shorter telomeres compared with the normal tissue despite the fact that most tumors express telomerase, the enzyme catalyzing the addition of telomeric repeats (4). In recent years, several studies have reported significant differences in blood telomere length between cancer patients and controls (5–13). Short blood telomere length has been suggested as a predisposition factor for, e.g., human bladder (5, 6), head and neck (5), lung (5, 7), and renal cell cancers (5, 8). In a recent study on skin cancer, long blood telomere length was associated with an increased melanoma risk but a decreased risk of basal-cell carcinoma (9). Barwell and colleagues (10) found no differences in blood telomere length between breast cancer patients and controls, whereas another study in sister sets reported a nonsignificant association between short telomere length and increased cancer risk (11). In contrast, we recently reported longer blood telomeres in newly diagnosed breast cancer patients with spontaneous tumors (12). Furthermore, we found that breast cancer patients with long blood telomere length (more than the median) had a significantly worse prognosis compared with patients with shorter telomeres, indicating a role for blood telomere length as a prognostic biomarker in breast cancer.

Renal cell carcinoma (RCC) accounts for ~3% of adult cancers. The disease is associated with a poor prognosis and nearly one third of the patients have metastasis present at diagnosis (14). To date, there are no ideal biomarkers for detection and/or prognostication of RCC, although a number of potential molecular markers have been investigated (15).

In the present study, we investigated whether blood telomere length may serve as a prognostic indicator in patients with newly diagnosed clear cell RCC (ccRCC), the most common RCC type (16). We also analyzed tumor and corresponding nonmalignant kidney cortex tissue to evaluate if telomere length in any of these compartments could predict survival. In addition, correlation analysis was performed between telomere length values of the three tissue compartments and various clinical variables. Relative telomere length (RTL) was measured by real-time PCR in peripheral blood, tumor tissue, and kidney cortex tissue of 105 patients with ccRCC. Interestingly, in the group without distant metastasis, patients with long blood RTL (fourth quartile) had a significantly worse outcome compared with patients with shorter blood RTL. In contrast, RTL in tumor or nonmalignant kidney cortex did not predict survival.

Materials and Methods

Patients. The patients were nephrectomized with histologically verified RCC at the Department of Urology, Umeå University Hospital, Umeå, Sweden. In total, 105 patients diagnosed between 2001 and 2007 were included in the study. There were 61 men (age range, 35–87; median age, 65) and 44 women (age range, 32–84; median age, 65). Routine staging procedures included physical examination and computerized tomography of the abdomen and chest. Additional investigations were made when indicated. Staging was performed according to the 2002 tumor-node-metastasis (TNM) classification system (17). Nuclear grading was performed according to Fuhrman and colleagues (18). RCC type was defined according to the Heidelberg consensus conference (16). Tumor size was measured on the surgical specimens and/or by computerized tomography. The tumors varied in size from 1.6 to 19 cm (median, 7.5 cm). Patients were followed-up with clinical and radiological examinations. Survival data were available for 103 patients. Of these, 28

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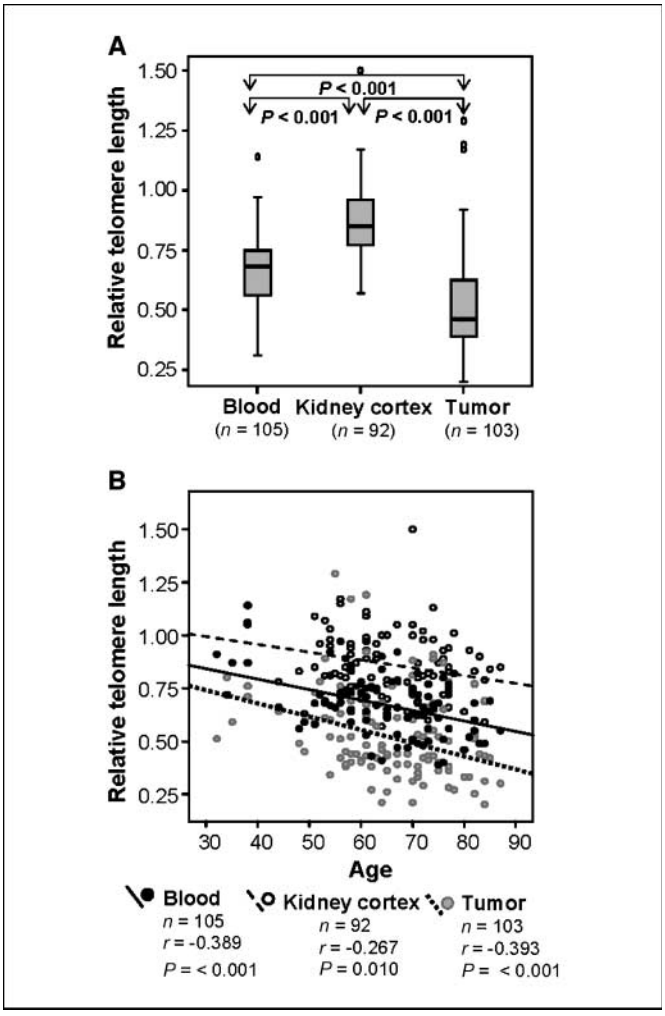

Figure 1. RTL in blood, kidney cortex, and tumor tissues of patients with clear cell renal cell carcinoma (ccRCC). *A,* box plots showing the minimum, lower quartile, median, upper quartile, and maximum RTL values. Mean values were compared by independent samples *t* test. *B,* correlation between RTL and age investigated using Pearson's correlation coefficient.

had died of the disease, 4 of other causes, and 71 patients were alive with a median survival of 24 mo (range, 5–78 mo). The study was approved by the regional ethical review board in Umeå (Dnr 07-071M), and each patient participated after providing informed and signed consent.

Tissue samples. Samples from tumors and kidney cortex, excised furthest away from the tumor mass, were obtained immediately after extirpation, snap-frozen in liquid nitrogen, and stored in −80°C until analysis. One part of each sample was also used for conventional histopathologic examination. Blood samples were collected prior to any therapy and buffy coats were frozen in −80°C until further analysis.

Real-time PCR for telomere length measurements. DNA was extracted from buffy coats (n = 105) and from freshly frozen tumor (n = 103) and kidney cortex samples (n = 92) using the BioRobot M48 Workstation with MagAttract technology as described elsewhere (Qiagen). Telomere length was assessed by real-time PCR as previously described (19, 20). Tumor and kidney cortex samples were tested mixed within the same plates. Blood telomere length was measured separately but with the same reagents. Telomere and β-globin primer sequences written 5′ to 3′ were CGGTTTGTTTGGGTTTGGGT-TTGGGTTTGGGTTTGGGTT (Tel 1b), GGCTTGCCTTACCCTTACCCTTACCC-TTACCCTTACCCT (Tel 2b), TGTGCTGGCCCATCACTTTG (HBG3), and ACCAGCCA-CCACTTTCTGA-TAGG (HBG4). T/S values were determined using the formula $T/S = 2^{-\Delta Ct}$, where ΔCt = average $Ct_{telomere}$ − average $Ct_{\beta\text{-globin}}$. RTL values were obtained by dividing sample T/S values with the T/S value of a reference sample (CCRF-CEM DNA), which was included in each run and loaded in the same amount as the unknown samples (17.5 ng). The reference DNA was also used to produce a standard curve for each plate (range, 3.7–50 ng) to monitor the PCR efficiency. All samples were loaded as triplicates. As a measurement of the intra-assay variability, the T/S value of the 17.7 ng standard curve point was compared with the T/S value of the reference sample. The mean intra-assay CV for 18 assays was 3.38%. The mean RTL value (±SD) for the 17.7 ng standard curve point was 1.01 ± 0.06, giving an inter-assay CV of 6.42%. In addition, 24 patient samples were run at two occasions with ∼3 mo interval. Plotting the RTL values of the two runs against each other generated an R^2 value of 94%. The mean CV for these samples was 7.05%.

Statistical analysis. SPSS version 15.0 was used for statistical analysis. Logarithmic transformations were applied to variables with slightly skewed distributions, so that tests requiring normality could be correctly performed. RTL means were compared by two-tailed unpaired and paired *t* tests or by analysis of covariance with age adjustments when appropriate. Pearson's correlation coefficient was used to calculate the correlation between telomere length and various continuous variables. Survival analysis was performed using Kaplan-Meier with the log-rank test and restricted to

Table 1. Correlation estimates between continuous clinical variables and RTL in blood, tumors and kidney cortex, investigated by Pearson's correlation analysis

	Blood RTL	Non-tumor RTL	Tumor RTL	Age	Tumor ø	ESR	Hemoglobin	TPC	Albumin
Blood RTL	1								
Non-tumor RTL	0.440*	1							
Tumor RTL	0.270*	0.449*	1						
Age	−0.389*	−0.267 †	−0.393*	1					
Tumor ø	−0.091	−0.079	0.238 †	−0.009	1				
ESR	−0.107	−0.159	−0.033	0.095	0.372*	1			
Hemoglobin	−0.042	0.002	−0.061	−0.028	−0.314*	−0.754*	1		
TPC	0.124	0.125	0.160	−0.142	0.287*	0.582*	−0.541*	1	
Albumin	0.099	0.075	−0.026	−0.178	−0.203 †	−0.595*	0.586*	−0.453*	1

Abbreviation: TPC, thrombocyte particle count.
*Correlation is significant at the 0.01 level (two-tailed).
†Correlation is significant at the 0.05 level (two-tailed).

Table 2. Kaplan-Meier survival analysis for prognostic factors in ccRCC, restricted to patients diagnosed before 2007

Variables	Total	Deaths	*P*	Valid cases	Missing cases
TNM stage				83	1
Stage I-III	56	5	<0.001		
Stage IV	27	22			
TNM T				83	1
T_1-T_2	48	9	0.001		
T_3-T_4	35	18			
TNM M				83	1
M_0	56	5	<0.001		
M_1	27	22			
Grade				82	2
Grades 1–3	64	14	<0.001		
Grade 4	18	12			
Capsule involvement				69	15
No	45	10	0.003		
Yes	24	13			
Anemia*				83	1
No	50	6	<0.001		
Yes	33	21			
Elevated ESR†				58	26
No	25	1	<0.001		
Yes	33	17			
Hypercalcemia‡				81	3
No	72	21	0.003		
Yes	9	6			
Thrombocytosis§				82	2
No	63	12	<0.001		
Yes	19	15			

NOTE: Groups were compared using the log-rank test.
*Cutoff for anemia: hemoglobin <134 for men and <117 for women.
†Cutoff for elevated ESR, >19 for men and >27 for women.
‡Cutoff for hypercalcemia: corrected S-calcium >2.60.
§Cutoff for thrombocytosis: thrombocyte particle count >348 for men and >387 for women.

patients diagnosed before 2007 (83 valid cases), to achieve adequate follow-up times. Survival was defined as the time (in months) between the date of diagnosis to ccRCC specific death or to the date of last follow-up (March 2008). Hazard ratios were calculated by multivariate Cox regression analysis. Statistical significance refers to $P \leq 0.05$ (two-tailed).

Results

Telomere length in blood, normal kidney, and tumor tissue. There was no difference in blood telomere length between men and women [age-adjusted mean RTL with 95% confidence interval (CI); 0.65 (0.62–0.69) and 0.65 (0.61–0.69), respectively; $P = 0.970$]. The mean RTL in nonmalignant kidney cortex was shorter in men (0.82; 95% CI, 0.78–0.86) compared with women (0.89; 95% CI, 0.84–0.94; $P = 0.04$), whereas no difference was seen in the tumors (men, 0.48; 95% CI, 0.44–0.52; women, 0.50; 95% CI, 0.45–0.55; $P = 0.610$; not shown in figures).

As Fig. 1*A* illustrates, tumors had significantly shorter telomeres compared with kidney cortex and blood. Also, telomeres in the kidney cortex were significantly longer compared with telomeres in the blood. Comparison of matched tissues generated the same results, i.e., RTL in kidney cortex > blood > tumor ($P < 0.001$). RTL data were available for 90 matching tumor and kidney cortex samples. Of these, 84 patients (93%) had shorter telomeres in the tumor compared with the kidney cortex [i.e., a tumor/non-tumor (T/N) RTL ratio <1.0], whereas 6 patients (7%) had longer telomeres in the tumor. Four (67%) of the latter six patients had metastatic disease, whereas 21 (25%) of the 84 patients with a T/N RTL ratio of <1.0 had metastasis.

Correlation investigations. Telomere length was inversely correlated with age in all tissue compartments analyzed (Fig. 1*B*). A summary of correlation investigations between RTL values and various continuous clinical variables can be viewed in Table 1, showing Pearson's correlation coefficients. Blood RTL, tumor RTL, and RTL in normal kidney were positively correlated with each other, but no correlations were found between RTL values and any of the clinical variables included [erythrocyte sedimentation rate (ESR), hemoglobin, thrombocyte particle count, and albumin]. There was, however, a positive correlation between tumor RTL and tumor size. A similar result was obtained when investigating the correlation between T/N RTL ratio and tumor size ($r = 0.360$, $P < 0.001$; not shown in figure).

Survival analysis. Table 2 shows survival analysis data for established prognostic variables. High TNM stage/distant metastasis (M_1), high nuclear grade, renal capsule invasion, anemia, elevated ESR, hypercalcemia, and thrombocytosis were all significantly associated with poor survival. There were no

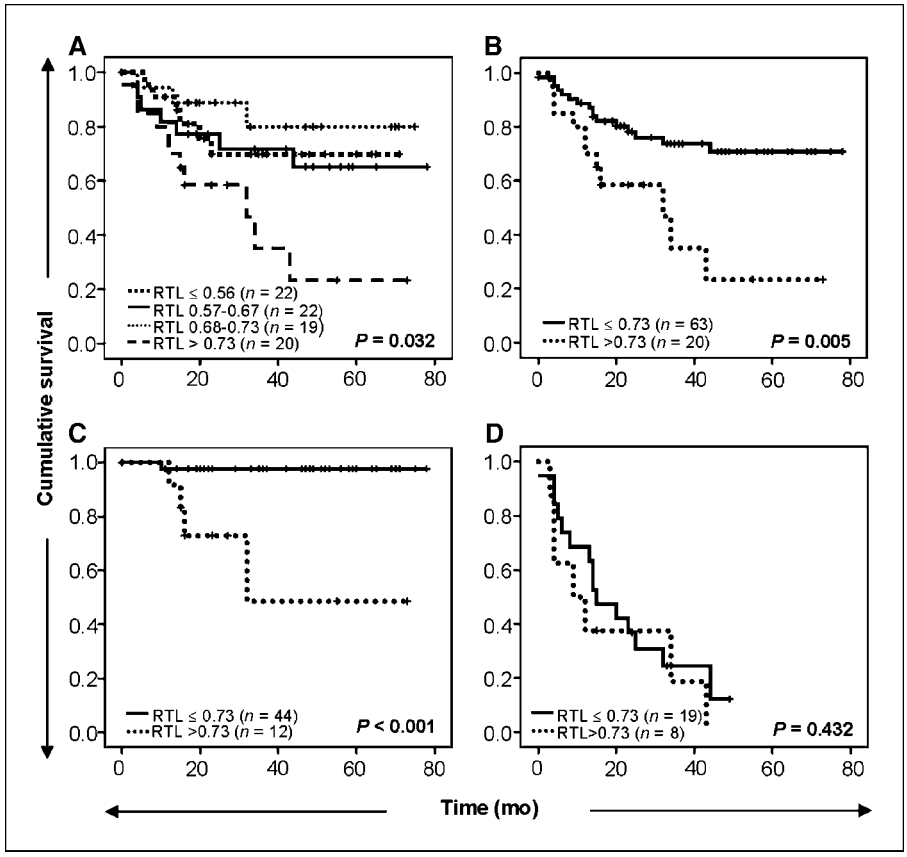

Figure 2. Kaplan-Meier survival analysis with the log-rank test, comparing patients with long vs. short blood telomere length. *A* and *B,* survival analysis for patients diagnosed from 2001 to 2006 (83 valid cases). *A,* all RTL quartiles compared. *B,* fourth RTL quartile (RTL > 0.73) vs. the first to third quartiles (RTL ≤ 0.73). *C,* survival analysis (fourth RTL quartile vs. first to third quartiles) restricted to patients with nonmetastatic disease ($n = 56$). *D,* survival analysis (fourth RTL quartile vs. first to third quartiles) restricted to patients with distant metastasis ($n = 27$).

significant differences in survival comparing patients ages ≤65 versus >65 years (median age; $P = 0.197$) or gender ($P = 0.085$, with a trend to longer survival in women; not shown in table).

To investigate whether blood telomere length carried any prognostic information, patients were divided into quartiles based on the RTL distribution. Patients with the longest blood telomeres (fourth quartile) had a significantly worse prognosis compared with patients with shorter telomeres (Fig. 2*A* and *B*). Analysis restricted to patients ages ≤65 and >65 years, respectively, showed that patients in the fourth quartile had a poorer prognosis in both age groups ($P = 0.034$ and $P = 0.048$, respectively; not shown in figure).

In the patient group without distant metastasis (M_0), a highly significant association ($P < 0.001$) was found between long blood RTL and poor outcome (Fig. 2*C*). A similar pattern (not shown in figure) was observed when restricting the analysis to patients without capsule involvement ($P = 0.001$) and patients having tumors with a nuclear grade of 1 to 3 ($P = 0.006$). In contrast, in patients with distant metastasis, the prognosis was poor regardless of the blood RTL (Fig. 2*D*). The same was true for patients with grade 4 tumors ($P = 0.525$) and for patients with renal capsule invasion ($P = 0.372$; not shown in figures). Multivariate Cox regression analysis, including age, blood RTL, and TNM stage, verified long blood RTL (RTL > 0.73) as an independent negative prognostic factor (Table 3). A similar result (not shown in table) was obtained when including hemoglobin (anemic versus non-anemic) as a variable instead of TNM stage (blood RTL, >0.73; hazard ratio, 2.8; 95% CI, 1.3–6.0).

Table 3. Multivariate Cox regression survival analysis, including age, blood telomere length, and TNM stage

Variables	Hazard ratio (95% CI)	*P*
Age (continuous)	0.9 (0.9–1.0)	0.021
Blood RTL		
RTL ≤ 0.73	1.0	
RTL > 0.73	3.0 (1.3–6.8)	0.008
TNM stage		
Stage I–III	1.0	
Stage IV	17.0 (6.1–47.0)	<0.001

NOTE: Eighty-three patients and 27 events were included.

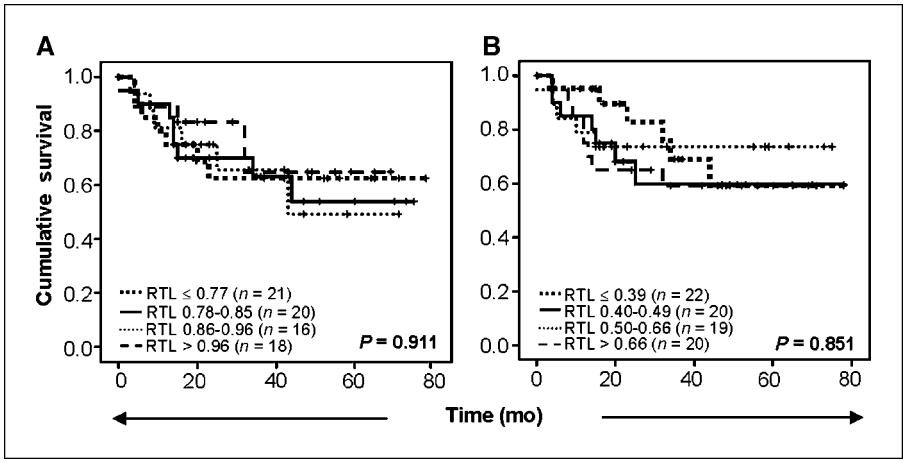

Figure 3. Kaplan-Meier survival analysis with the log-rank test, comparing non-tumor and tumor RTL quartiles, respectively. *A,* survival comparison between non-tumor RTL quartiles (n = 75). *B,* survival comparison between tumor RTL quartiles (n = 81).

In contrast to blood RTL, RTL in kidney cortex tissue or ccRCC did not predict survival time when comparing patients in different RTL quartiles (Fig. 3*A* and *B*). A similarly nonsignificant pattern in kidney cortex or tumor tissue was observed for M_0 patients as for M_1 patients (not shown in figure). When the patients were subdivided into two groups based on the median T/N RTL ratio (0.55), a nonsignificant (P = 0.086) trend to shorter survival was noted in patients with a high T/N RTL ratio (not shown in figure).

Discussion

The present study is the first to investigate RTL in three different compartments of patients with ccRCC: peripheral blood, nonmalignant kidney cortex, and ccRCC. As expected, the shortest telomeres were found in the tumors. Furthermore, telomeres in the kidney cortex were significantly longer compared with peripheral blood, suggesting differences in the replicative history of the cells and/or differences in telomere length regulation. Previous studies have shown a correlation between telomere length in different organs in both humans and monkeys (21, 22), which was supported by our data on blood and nonmalignant kidney tissue. In addition, we found a surprising correlation between RTL in these compartments and tumor tissue. Tumors, besides having the shortest telomeres overall, also exhibited an age-associated decline in RTL similar to blood and kidney cortex tissue. Hence, ccRCCs in younger patients displayed longer telomeres compared with tumors in older patients. These data might suggest that telomere length per se is not part of the etiology of ccRCC, i.e., transformation does not occur at a certain critically short telomere length.

The most striking finding in our study is that blood RTL could predict survival for patients with ccRCC. This finding is in line with our previous observation in breast cancer (12), and further substantiates blood RTL as a significant biological marker. The fact that RTL in blood, but not in normal kidney or in tumor tissue, was associated with prognosis suggests a systemic effect on the hematopoietic system. In our previous study, in which patients with breast cancer with long blood RTL had a worse prognosis, we speculated that this feature might reflect a combined effect of epigenetic regulation, cytokines, and hormones. The response of the immune system in cancer is very complex. There is evidence that RCC can be recognized by immune cells, and a small subset of patients with RCC respond well to immunotherapy (23). Tumors, however, can evolve mechanisms to escape the immune attack and several tumor-associated suppressive mechanisms have been described in RCC, e.g., alterations in lymphocyte reactivity and modulation of immune cell infiltration and activity (23). Regulatory T cells (Treg cells), a subpopulation of $CD4^+$ T cells, also seem capable of inhibiting antitumor immune responses (23, 24). Increased levels of Treg cells have been detected in blood and at the tumor site of patients with cancer (25–29). Down-regulation of the immune response in a subset of cancer patients, caused by Treg cells and/or other immunomodulators, could theoretically lead to less telomere attrition due to fewer cell divisions, and to a reduced antitumoral activity. Such a theory might explain our observations that long blood telomeres in ccRCC, as well as in patients with breast cancer, were associated with a worse outcome. In addition, as discussed previously (12), it cannot be excluded that a subset of patients with long telomeres had elevated levels of various telomerase-stimulating factors with effects on the hematopoietic system. More detailed analyses of blood constituents and tumors are needed in the future, in order to identify possible immune-related differences between patients with long versus short blood RTL. Examples of such theoretically relevant differential factors are serum cytokines with the potential to regulate telomerase and immunomodulating Treg cells. The overall cell subset composition in the blood may also differ and further analyses of the telomere biology of specific blood cell subsets in tumor patients are warranted. Tumor-directed analyses are also of interest because individual differences in the expression and release of proteins with secondary effects on blood RTL cannot be excluded.

Blood RTL was found to be a significant prognostic indicator in patients with nonmetastatic disease. The outcome for RCC patients with metastasis is, in general, poor, and blood RTL could not predict survival in these patients. The statistics regarding this subgroup should, however, be taken with caution due to the rather small number of individuals. Of special interest is the finding that RTL was unrelated to other well-established prognostic indicators such as hemoglobin, ESR, thrombocyte count, and albumin. All these factors are coupled to more advanced disease whereas blood RTL might be helpful in identifying patients with local disease, but with a high risk for relapse and a poor outcome. This patient subgroup might benefit from new therapy strategies. Hence, prospective treatment programs taking blood RTL into account could be an interesting option. Identifying patients with less aggressive disease is also important because overtreatment with unnecessary side effects might then be avoided. Our finding that

the M_0 subgroup with shorter telomeres had a good long-term survival is therefore of interest. Based on our previously published data, the same arguments are valid for breast cancer, and future studies will show if blood RTL is a more general prognostic indicator for additional tumor types. Therefore, extended studies on patients with other cancer forms are needed. In order to investigate the importance of blood RTL in different patient subgroups, studies encompassing a larger sample size, including a larger number of patients with metastatic disease, are warranted.

In contrast, RTL in tumor and kidney cortex tissue did not predict survival. It might seem inconsistent that only blood RTL predicted survival, when at the same time, a positive RTL correlation was found between blood, tumor, and kidney cortex tissues. However, although statistically significant, the correlations between the different tissues were not very strong. As an example, the correlation coefficient between blood and nonmalignant kidney cortex was $r = 0.440$, giving an r-squared value of 0.194, or $\sim$19%. Hence, although the tissues correlated positively with each other regarding RTL, the correlation was far from 100% and different factors are likely to influence telomere length maintenance in different tissue compartments. As has been speculated above, it might be that the cancer disease itself causes a systemic/immunorelated effect on the telomere length of peripheral blood.

We did find a positive correlation between tumor size and tumor RTL and between tumor size and T/N RTL ratio. In addition, there was a nonsignificant trend of worse survival in patients with a high T/N RTL ratio. Also, four of the six patients with a T/N RTL ratio of >1.0 had distant metastasis, as compared with one fourth of the patients with smaller ratios. Several studies have reported longer telomeres in tumors of more advanced stages, e.g., in Barrett carcinoma (30), hepatocellular carcinoma (31), and colorectal carcinoma (32, 33). In these studies, a high T/N RTL ratio was associated with a worse prognosis. Oh and colleagues (31) reported that advanced hepatocellular carcinomas with poor outcome showed high telomerase activity and long telomeres. In colorectal cancer, patients with telomerase-positive tumors and a high T/N RTL ratio had a significantly shorter disease-free survival compared with patients with lower RTL ratios (33). We did not have data on telomerase activity in the present study, but our finding of a trend to shorter survival in patients with a high T/N RTL ratio supports the previous studies. It may well be that long tumor telomere length reflects telomere stabilization important for tumor progression.

In conclusion, the data presented here show that blood RTL was a significant independent prognostic indicator in ccRCC. In contrast, RTL quartiles in tumor and nonmalignant kidney cortex did not predict survival. Importantly, blood RTL was independent of established prognostic indicators and could predict survival in ccRCC patients with limited disease. This may be of importance for future treatment strategies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

1. Chan SR, Blackburn EH. Telomeres and telomerase. Philos Trans R Soc Lond B Biol Sci 2004;359:109–21.
2. Harley CB. Human ageing and telomeres. Ciba Found Symp 1997;211:129–39.
3. Hacket JA, Greider CW. Balancing instability: dual roles for telomerase and telomere dysfunction in tumorigenesis. Oncogene 2002;21:619–26.
4. Cong YS, Wright WE, Shay JW. Human telomerase and its regulation. Microbiol Mol Biol Rev 2002;66:407–25.
5. Wu X, Amos CI, Zhu Y, et al. Telomere dysfunction: a potential cancer predisposition factor. J Natl Cancer Inst 2003;95:1211–8.
6. McGrath M, Wong JY, Michaud D, Hunter DJ, De Vivo I. Telomere length, cigarette smoking, and bladder cancer risk in men and women. Cancer Epidemiol Biomarkers Prev 2007;16:815–9.
7. Jang JS, Choi YY, Lee WK, et al. Telomere length and the risk of lung cancer. Cancer Sci 2008;99:1385–9.
8. Shao L, Wood CG, Zhang D, et al. Telomere dysfunction in peripheral lymphocytes as a potential predisposition factor for renal cancer. J Urol 2007;178:1492–6.
9. Han J, Qureshi AA, Prescott J, et al. A prospective study of telomere length and the risk of skin cancer. J Invest Dermatol 2009;129:415–21.
10. Barwell J, Pangon L, Georgiou A, et al. Is telomere length in peripheral blood lymphocytes correlated with cancer susceptibility or radiosensitivity? Br J Cancer 2007;97:1696–700.
11. Shen J, Terry MB, Gurvich I, Liao Y, Senie RT, Santella RM. Short telomere length and breast cancer risk: a study in sister sets. Cancer Res 2007;67:5538–44.
12. Svenson U, Nordfjäll K, Stegmayr B, et al. Breast cancer survival is associated with telomere length in peripheral blood cells. Cancer Res 2008;68:3618–23.
13. Risques RA, Vaughan TL, Li X, et al. Leukocyte telomere length predicts cancer risk in Barrett's esophagus. Cancer Epidemiol Biomarkers Prev 2007;16:2649–55.
14. Motzer RJ, Bander NH, Nanus DM. Renal-cell carcinoma. N Engl J Med 1996;335:865–75.
15. Tunuguntla HS, Jorda M. Diagnostic and prognostic molecular markers in renal cell carcinoma. J Urol 2008;179:2096–102.
16. Kovacs G, Akhtar M, Beckwith BJ, et al. The Heidelberg classification of renal cell tumours. J Pathol 1997;183:131–3.
17. Sobin LH, Wittekind CH. Eds: International Union Against Cancer (UICC). TNM classification of malignant tumours. 6th ed. New York: Wiley-Liss; 2002. p. 193–5.
18. Fuhrman SA, Lasky LC, Limas C. Prognostic significance of morphologic parameters in renal cell carcinoma. Am J Surg Pathol 1982;6:655–63.
19. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002;30:e47.
20. Nordfjäll K, Osterman P, Melander O, Nilsson P, Roos G. hTERT (−1327)T/C polymorphism is not associated with age-related telomere attrition in peripheral blood. Biochem Biophys Res Commun 2007;358:215–18.
21. Friedrich U, Griese E, Schwab M, Fritz P, Thon K, Klotz U. Telomere length in different tissues of elderly patients. Mech Ageing Dev 2000;119:89–99.
22. Gardner JP, Kimura M, Chai W, et al. Telomere dynamics in macaques and humans. J Gerontol A Biol Sci Med Sci 2007;62:367–74.
23. Frankenberger B, Noessner E, Schendel DJ. Immune suppression in renal cell carcinoma. Semin Cancer Biol 2007;17:330–43.
24. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008;133:775–87.
25. Liyanage UK, Moore TT, Joo HG, et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. J Immunol 2002;169:2756–61.
26. Curiel TJ, Coukos G, Zou L, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004;10:942–9.
27. Viguier M, Lemaître F, Verola O, et al. Foxp3 expressing CD4+CD25(high) regulatory T cells are overrepresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells. J Immunol 2004;173:1444–53.
28. Ormandy LA, Hillemann T, Wedemeyer H, et al. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. Cancer Res 2005;65:2457–64.
29. Hirokawa K, Utsuyama M, Ishikawa T, et al. Decline in T cell-related immune functions in cancer patients and an attempt to restore them through infusion of activated autologous T cells. Mech Ageing Dev 2009;130:86–91.
30. Gertler R, Doll D, Maak M, Feith M, Rosenberg R. Telomere length and telomerase subunits as diagnostic and prognostic biomarkers in Barrett carcinoma. Cancer 2008;112:2173–80.
31. Oh BK, Kim H, Park YN, et al. High telomerase activity and long telomeres in advanced hepatocellular carcinomas with poor prognosis. Lab Invest 2008;88:144–52.
32. Gertler R, Rosenberg R, Stricker D, et al. Telomere length and human telomerase reverse transcriptase expression as markers for progression and prognosis of colorectal carcinoma. J Clin Oncol 2004;22:1807–14.
33. Garcia-Aranda C, de Juan C, Diaz-Lopez A, et al. Correlations of telomere length, telomerase activity, and telomeric-repeat binding factor 1 expression in colorectal carcinoma. Cancer 2006;106:541–51.