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Prognostic Relevance of hTERT mRNA Expression in Ductal Adenocarcinoma of the Pancreas[1,2]

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Abstract

Telomerase is thought to play an essential role in tumorigenesis and progression. Its activity is directly correlated with the expression of its catalytic subunit, human telomerase reverse transcriptase (hTERT). A correlation of transcript expression with a poor prognosis has been detected in different human malignancies. However, data on hTERT in pancreatic ductal adenocarcinoma (PDAC) are purely descriptive so far. Therefore, we evaluated the impact of hTERT expression on patients' prognosis. Human telomerase reverse transcriptase mRNA isolates from 56 human microdissected PDAC tissues were analyzed by quantitative reverse transcription–polymerase chain reaction and multivariate Cox regression hazard test. Elevated hTERT transcript levels were measured in 23 of 56 PDAC tissues, 33 patients showed no detectable transcripts. Unexpectedly, a low expression of hTERT mRNA levels was associated with a worse prognosis for overall survival (relative risk = 5.33; $P = .013$) when compared to high levels, whereas undetectable expression showed an intermediate risk of tumor-related death. These data challenge previous findings outlining hTERT's negative impact on overall survival. The risk pattern obtained in PDAC suggests a more complex regulation of hTERT.

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Introduction

Current molecular oncology strongly suggests that aberrant reactivation of telomerase is one of the key features of the malignant phenotype of a somatic cell [1–4]. Telomerase catalyzes the synthesis and extension of telomeric DNA, thus leading to inactivation of apoptosis and senescence [3,5]. To detect and measure telomerase activity, the mRNA expression of its catalytic subunit human telomerase reverse transcriptase (hTERT) has been proposed as a surrogate marker, given the fact that mRNA levels correlate directly with telomerase activity [6,7]. Data on the clinical relevance of hTERT expression in malignant neoplasias are yet irresolute, although hTERT expression levels suggest a correlation with a poor prognosis in non–small cell lung cancer, Wilm tumor, B-chronic lymphocytic leukemia, acute myelogenous leukemia, colorectal cancer [8–10], and in soft-tissue sarcoma [11,12].

Pancreatic ductal adenocarcinoma (PDAC) is a dismal disease because of its aggressive biologic phenotype, characterized by an early local invasion and high metastatic potential, late clinical presentation, very poor overall prognosis with a short median survival time of only a few months after diagnosis (ranging from a few weeks to years), and high resistance to radiation and chemotherapy [13].

Most recently, telomerase and hTERT activity have been reported in tumors and pancreatic juice of patients with PDAC [14–18], and

Abbreviations: HPRT, hypoxanthine-phospho-ribosyl-transferase; hTERT, human telomerase reverse transcriptase; PDAC, pancreatic ductal adenocarcinoma; RR, relative risk

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their possible role in early diagnosis has been proposed. So far, there are no studies on the impact of hTERT expression on the prognosis of patients with PDAC. Therefore, we investigated precisely this in a cohort of 56 PDAC patients from our institution. We microdissected fresh-frozen tumor tissues to highly enrich neoplastic cells, isolated mRNA, and applied a quantitative polymerase chain reaction analysis for hTERT message. The impact of the gene expression on prognosis was determined by a multivariate Cox regression hazard model.

Materials and Methods

Patients

The study comprised a cohort of 56 patients (20 females and 36 males; age range, 34-80 years; mean age, 61.7 years), who were monitored for a mean observation time of 15.8 months (range, 1-61 months) and whose median survival rate was 14 months (range, 1-49 months). All patients included in the study underwent primary surgery in the years 2001–2005 at our hospital (Department of Surgery 1, University of Ulm, Ulm, Germany). Surgical pancreatic resection specimens were immediately placed on ice and subsequently snap-frozen and stored at –80°C. All patients gave written informed consent, and approval of the ethics committee was obtained.

Microdissection

RNA was isolated using the Innuprep RNA mini-kit (AJ Innuscreen GmbH, Berlin, Germany). The integrity of the isolated RNA was confirmed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The cryostat tissues were cut into 8- to 10-μm sections. Microdissection of selected areas containing approximately 50 to 300 neoplastic ductal epithelial cells per area (>4000 cells per tissue per patient) was carried out by means of laser microdissection and pressure catapulting (LCM) technique (PALM Microlaser Technologies, Bernried, Germany) on cresyl violet–stained sections.

Quantitative Reverse Transcription–Polymerase Chain Reaction

Measurement of hTERT transcript expression levels was performed by quantitative real-time reverse transcription–polymerase chain reaction as described previously [11,12]. The cutoff levels for low expression of hTERT were set at 0.00 ag/fg hypoxanthine-phospho-ribosyl-transferase (HPRT), whereas the cutoff for high expression was set at the mean value of hTERT expression (Table W1). Accordingly, three categories of expression levels were set up: 1, "no expression" (meaning no detectable mRNA levels); 2, "low expression" (>0.00 and ≤61 ag hTERT mRNA/fg HPRT mRNA); 3, "high expression" (>61 ag hTERT/fg HPRT mRNA). Additionally, the "low-expression" and "high-expression" groups were merged into a joint group comprising patients with detectable hTERT levels (>0.00 ag hTERT/fg HPRT mRNA).

Statistical Analysis

Multivariate analysis according to Cox proportional hazards regression model (with adjustment for tumor staging, type of tumor resection, and patients' age) was performed for the analysis of hTERT mRNA expression. For statistical analyses, SPSS 15.0 software was used. $P < .05$ was considered significant.

Results

Of our cohort, 33 had undetectable, 17 had low, and 6 had high hTERT mRNA levels. The clinical data of the resulting three subgroups are listed in Table 1. The data on the impact of the gene expression on prognosis—as determined by a multivariate Cox regression hazard model—are plotted in Figure 1.

Surprisingly, the analysis of patients of all stages and resection types showed that patients with low expression levels of hTERT message in their PDAC did worse than those with high hTERT mRNA [relative risk (RR) = 3.39; $P = .046$; Figure 1]. Unexpectedly again, patients with undetectable hTERT took an intermediate course (RR = 1.60; $P = .392$) when compared with patients presenting high hTERT levels. Aiming at the analysis of patients treated with a curative intent, we excluded patients with advanced disease (UICC stage IVb—concomitance of metastases and/or local R2-resection). Within this group of patients, the presence of metastases and/or nonresectable tumor mass might be a more decisive factor of survival than the expression of hTERT. Interestingly, on such analysis, an even more explicit, significant association of a worse prognosis in patients with low expression of hTERT was observed (RR = 5.33; $P = .013$) when compared to patients with a high expression profile.

Table 1. Clinical and Histopathologic Data.

	Total (n = 56)	Low hTERT (n = 17)	Not Detectable hTERT (n = 33)	High hTERT (n = 6)	Patients at Follow-up	
					Alive* (n = 6)	Dead† (n = 50)
Men/Women	36:20	11:6	20:13	5:1	4:2	32:18
Tumor stage						
I	2	1	1	0	1	1
II	12	2	8	2	1	11
III	31	7	21	3	4	27
IV	11	7	3	1	0	11
Tumor resection						
Radical (R0)	38	9	25	4	5	33
Not radical (R1)	9	2	6	1	1	8
Not radical (R2)	9	6	2	1	0	9
Patients at follow-up						
Alive*	6	0	5	1	6	
Dead†	50	17	28	5		50

Data are number of patients.

*After an average observation time of 15.8 months (range, 1-61 months).

†Patients died after an average of 14 months (range, 1-49 months), 2 patients died of non–tumor-related reasons.

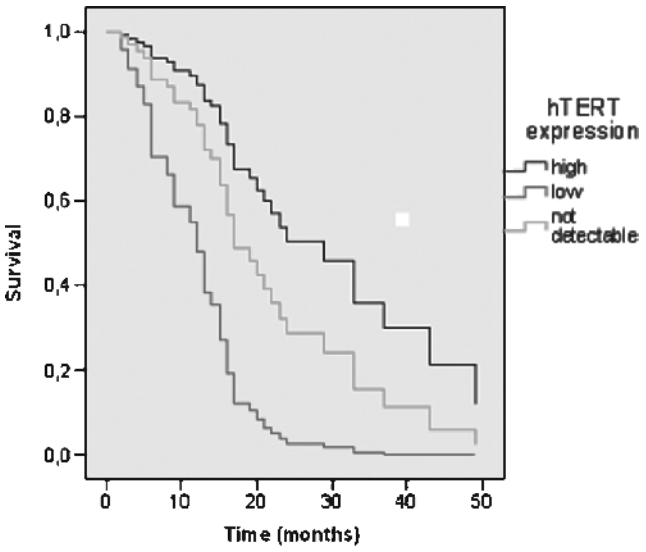

Figure 1. Prognostic relevance of hTERT mRNA expression, analyzed with multivariate Cox proportional hazard regression model for overall survival for 56 PDAC patients (UICC stages I-IV, R0-2 resection), adjusted for tumor stage, type of tumor resection, and patients' age. Cutoffs for expression of hTERT were the mean value (61 ag) and undetectable mRNA levels (0.00 ag): >61 ag (high); >0.00 to ≤61 ag (low), and 0.00 ag mRNA (not detectable) standardized to HPRT transcript levels.

Undetectable expression was again associated with a slightly worse outcome compared with patients with high hTERT levels (RR = 1.83; $P = .32$). Furthermore, in comparison with the patients showing undetectable expression of hTERT, the risk of tumor-related death in those with the low expression profile showed a highly significant increase to 3.8-fold ($P = .002$). Additionally, we analyzed the impact of the overall expression of hTERT (merged "low-expression" and "high-expression" groups; $n = 23$) on survival. The comparison of this group with patients presenting undetectable hTERT mRNA levels showed a tendency—however, not significant—for an overall increased risk of tumor-related death for patients expressing hTERT mRNA (RR = 1.358; $P = .340$).

Accordingly, analysis of the three hTERT expression profile groups with regard to the 18-month, and the average survival rate was performed. Of 33 patients with undetectable hTERT expression, 14 were still alive 18 months after diagnosis and surgery or had died within this time span of non–tumor-related causes. Interestingly, only 1 of 17 patients with low hTERT expression survived this period, whereas 3 of 6 patients with high hTERT expression were either still alive or had died due to non–tumor-related causes. The average survival rate in each group was 9.5 months in the low expression profile group, 18.2 months for patients with undetectable hTERT expression, and 20.8 months for patients with high hTERT expression.

Discussion

The data from this study challenge the present view that telomerase activity, strictly and in general, correlates with the biologic behavior of a malignant tumor when it comes to complex parameters such as survival after surgery. The expression of low levels of hTERT mRNA in PDAC does have a negative impact on patients' prognosis and a tendency for an association of hTERT expression in general with an unfavorable outcome can also be observed in our study. These data are at first consistent with observations made on other tumor entities. Among others, hTERT overexpression is (solely) associated with a significant poor outcome in soft-tissue sarcoma [11,12], non–small cell lung cancer [10] and also in combination with cytokeratins 19/20 and carcinoembryonic antigen in colorectal cancer [9]. However, the somewhat better prognosis of patients accompanying a high hTERT expression profile has not been reported for other malignant tumors up-to-date. This intriguing, more favorable outcome might indicate a complex and precise regulation of hTERT, affirming evidence on the complex biology of telomerase regulation.

Current evidence suggests that hTERT expression and its mRNA levels are mainly controlled at the level of transcription by such means as hypoxia (through hypoxia response element sites), mitogens, hormones (e.g., estrogen), chromatin remodeling, and cell signaling pathways [1,19]. Thus, hTERT's interrelation with different oncogenes, including *c-Myc*, *Bcl-2*, *Her-2*, *Ras* [20–22], *p53* [23], and *survivin* [11,12,24], and the transcription factors Sp1 and Sp3 [19] has been suggested to play an important role in cancer progression and telomerase control.

However, additional ways of the regulation of hTERT expression have been proposed and provide possible explanations for the observations in our study. Thus, regulation of transcript processing, changes in mRNA half-life [19], specific activation of hTERT promoter by enhancer binding protein-2β [25], or hTERT gene regulation in the form of alternative splicing (e.g., with the active wild type and inactive b variant, as shown under hypoxic conditions) [26–28] might account for low hTERT activity despite high mRNA levels and effectively result in a better prognosis for PDAC patients. Thus, some alternately spliced deletion variants of hTERT were shown to be functionally inactive or even impair the function of telomerase (e.g., the hTERTα$^-$ splice variant), causing telomere shortening and cell death [29,30]. Tumors with a high hTERT expression profile might have either less or inactive variants of its mRNA, be subject to faster degradation or ineffective translation processes, resulting in a less functional protein expression.

In summary, measurement of hTERT transcription levels identifies groups of PDAC patients with different prognoses, with a patient group showing a 5.33-fold increased risk of tumor-related death. This might have an important potential as a predictor of survival. The oncobiologic role of telomerase activity in PDAC, intriguingly, remains elusive so far.

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Table W1. Clinical and Histopathologic Data; Quantitative RT-PCR Measurements.

Patient No.	Age (years)	Survival (months)	Censor	Stages	R-Status	HPRT (fg/μl)	hTERT (ag/μl)	hTERT (ag/fg HPRT)
1	59	13	1	3	0	55.073	4551.100	82.638
2	53	16	1	3	0	955.129	13506.410	14.141
3	60	21	1	2	0	262.407	30491.030	116.197
4	64	39	2	2	1	1069.529	116479.350	108.907
5	40	19	1	3	0	7858.380	406902.390	51.779
6	59	24	1	3	0	231.136	0.000	0.000
7	47	20	1	3	0	386.544	0.000	0.000
8	46	15	1	3	0	173.775	0.000	0.000
9	71	5	1	2	1	373.211	0.000	0.000
10	66	4	1	4	2	27.898	1451.890	52.042
11	70	15	1	3	1	39.054	672.190	17.212
12	67	6	1	3	0	19.760	2330.960	117.963
13	74	3	1	4	2	363.078	10704.520	29.483
14	75	49	1	3	0	578.892	0.000	0.000
15	49	22	1	3	0	189.006	0.000	0.000
16	70	4	1	3	0	45.324	0.000	0.000
17	68	11	1	2	0	265.628	0.000	0.000
18	72	33	1	3	1	206.232	0.000	0.000
19	73	1	4	3	0	28.330	0.000	0.000
20	58	43	1	3	0	1163.919	86123.510	73.994
21	74	61	2	3	0	2410.464	0.000	0.000
22	69	15	1	4	2	149.650	2045.880	13.671
23	43	2	1	4	2	726.099	8015.350	11.039
24	56	5	1	4	2	682.589	0.000	0.000
25	48	9	1	3	0	38.549	0.000	0.000
26	70	8	1	4	2	493.055	14326.020	29.056
27	76	15	1	2	0	274.007	0.000	0.000
28	57	3	1	4	0	1474.944	1223.100	0.829
29	61	14	1	3	0	512.313	3354.430	6.548
30	69	9	1	3	0	262.491	12208.950	46.512
31	47	2	1	3	0	90.737	1961.580	21.618
32	52	29	1	2	0	467.628	0.000	0.000
33	38	4	1	4	2	138.001	0.000	0.000
34	65	16	1	2	0	105.754	6372.660	60.259
35	75	13	1	2	0	914.723	8537.610	9.334
36	60	4	1	4	1	943.189	0.000	0.000
37	61	37	1	3	0	466.807	0.000	0.000
38	62	16	1	2	1	136.162	0.000	0.000
39	80	33	1	3	0	92.629	0.000	0.000
40	69	1	4	3	0	343.182	0.000	0.000
41	42	3	1	4	2	259.850	114535.130	440.775
42	52	28	2	3	0	5.231	0.000	0.000
43	52	13	1	3	0	38.957	0.000	0.000
44	71	23	1	2	0	71.127	0.000	0.000
45	64	23	2	3	0	121.342	0.000	0.000
46	69	19	2	3	0	458.081	0.000	0.000
47	68	19	2	1	0	1453.104	0.000	0.000
48	34	6	1	3	1	943.603	54612.080	57.876
49	61	12	1	1	0	209.867	7199.790	34.306
50	71	12	1	2	0	28.472	0.000	0.000
51	66	17	1	3	0	129.289	0.000	0.000
52	74	17	1	2	0	584.660	0.000	0.000
53	67	17	1	3	0	29.757	0.000	0.000
54	67	6	1	3	1	192.573	0.000	0.000
55	69	5	1	4	2	465.172	6802.180	14.623
56	55	8	1	3	1	2734.667	0.000	0.000

Survival: postoperatively until follow-up or death.
Censor: *1* indicates died due to tumor; *2*, alive at follow-up (without recurrence or metastasis); *4*, died due to non–tumor-related causes.
Stages: *1* indicates stage I; *2*, stage II; *3*, stage III; *4*, stage IV.
R-Status—resection type: *0* indicates R0; *1*, R1; *2*, R2.