Telomerase Expression as a Marker in Prostate Cancer: Correlation to Clinicopathologic Predictors

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The aim of this study was to investigate by an *in situ* hybridization procedure the Telomerase expression as a marker in prostate cancer and to correlate these results with several prognostic factors concerning this cancer.
Imprint smear samples were obtained from 70 prostates removed from patients who underwent radical prostatectomy for prostate adenocarcinoma. Telomerase expression in cancerous prostate smears was studied using an *in situ* hybridization procedure. The results were correlated with prognostic factors such as pathologic staging, Gleason grading, PSA serum levels and tumour differentiation.
Positive Telomerase expression was detected in 88.6% prostate cancer smears. Telomerase expression was significantly correlated with the Gleason score ($p<0.001$), tumour differentiation ($p<0.001$) and PSA serum levels ($p=0.002$). The distribution of Telomerase expression according to histopathological staging was not statistically significant ($p<0.56$).
In conclusion Telomerase expression could be a marker indicating the malignant potential of prostate cancer.

Key Words: Telomerase, Prostate cancer, *in situ* hybridization, Prognostic factors

In the last years, a very strong association between the presence of Telomerase activity and malignancy has been reported, making this enzyme one of the most common tumour markers (1-6).

Several studies reported strong positive Telomerase activity in primary prostate cancer in the radical prostatectomy specimens in contrast to the normal prostatic tissues in which this activity has not been detected (7,8).

The aim of this study was to investigate by an *in situ* hybridization (ISH) procedure the Telomerase expression as a marker in prostate cancer and to correlate these results with several prognostic factors concerning this cancer.

Materials and Methods

Samples were obtained from 70 patients who underwent radical prostatectomy for prostatic adenocarcinoma. Patients' age ranged from 59 to 75 years (mean 67.11 years). Imprint smears were taken from different areas of macroscopically estimated prostatic cancer. All samples were obtained immediately after prostate removal at the operation theatre. After air drying, cytologic smears were fixed in ethanol/acetone 1:1 for 10 min and stored at -70°C until used by an ISH procedure.

The histopathological diagnoses were performed using sections from the same samples that were used for the imprints. The TNM system (based on the staging system of the American Joint Committee on Cancer) (8) was used for pathologic staging and grading of the primary cancer was evaluated according to the Gleason score system. None of the patients had been treated with radiation or androgen depletion prior to prostatectomy.

Follow up after surgery ranged from 6 to 36 months (mean 29.2 months) and included serum PSA at 6 months postoperatively and 12, 18, 24 and 36 months thereafter. The clinicopathological features of prostate cancer cases are displayed in Table I.

To date, 5 patients are dead because of prostatic adenocarcinoma (3 in one year, 1 in two years and 1 in six months). The remaining patients are alive and well at the last follow up.

For the detection of Telomerase RNA activity on smear cells an immunodetection system for *in situ* hybridization (ISH) was used (Bio-Genex San Ramon CA 94583). The smears were rinsed in phosphate

Table I - Clinical characteristics of 70 patients treated with radical prostatectomy

Data of the patients	No	(%)
Age (yrs)		
<65	14	20.0
65-69	35	50.0
>70	21	30.0
Location (lobe)		
Left	23	32.9
Right	47	67.1
Stage		
T2a	44	62.9
T2b	17	24.3
T2c	7	10.0
T3a	2	2.9
Gleason Score (grade)		
2-4	19	27.1
5-6	30	42.9
>7	21	30.0
Differentiation		
Poorly	60	85.7
Well	10	14.3
Pretreatment PSA levels		
0-5	13	18.6
5.1-9.9	36	51.4
>10	21	30.0

buffered saline (PBS) and then were treated with proteinase K in 100 mM Tris-HCI, 50 mM EDTA for 15 min at 37°C. Smears were rinsed in paraformaldehyde (4%) in PBS for 5 mins and acetylated in 0.25% acetic anhydrite, 0.1 triethanolocuine for 10 mins. Prior to hybridization the smears were dehydrated in gradually increasing concentrations (50%, 70%, 100%) of ethanol for 10 secs, respectively.

Smears were prehybridized for 60 mins at 42°C in hybridization buffer. Hybridization was performed at 42°C overnight in the hybridization buffer containing an 800 ng/ml probe. The smears were washed at 42°C in SSC x 2 for 5 mins, at 42°C in 50% formamide, 0.2xSSC for 5 mins and at 42°C in 0.2xSSC for 5 mins. They were then washed in 2 changes of 2xSSC for 5 mins each followed by one wash using 1xSSC for 5 mins, the slides were incubated in protein block (protein blocking reagents in PBS with 0.09% sodium acid) at room temperature for 20 mins. After 3 washes in 1xPBS for 5 mins, 1-2 drops of covered mouse antibiotin antibody were put on slides for 20 mins, after 3 washes 2 drops of biotinylated F2 fragment of anti-mouse immunoglobulins in PBS were put at room temperature for 30 mins and alkaline phosphatase was detected using 5-bromo-4-chloro-3-idolyl phosphate and nitro blue tetrasodium chloride at room temperature for 30 mins. Slides were then rinsed in 3 changes of distillated water and counterstained with eosin for 30 secs.

Smears incubated with the digoxigenin-labelled sense probe in the same conditions were used as negative controls. Smears of liver metastatic cells having strong Telomerase activity by the TRAP method were used as a positive control in our method.

Each slide was reviewed by two cytopathologists and a representative sample of 300 cells counted simultaneously for the presence or absence of staining which was noted in the nucleus of the malignant cells (Fig. 1, 2). Telomerase positivity was scored as follows: staining of <5% of cells(-), 5-25%(+), 26-74%(++), 75-100%(+++). Nucleus Telomerase staining was positive if >5% and negative if <5% of cells stained.

The relationship of Telomerase expression with all prognostic variables (stage, Gleason score, PSA serum levels and tumour differentiation) was assessed by one way analysis of variance (ANOVA) followed by tests of multiple comparisons since Telomerase did not deviate from normality (Kolmogorov-Smirnof test: p=0.281). The simultaneous effect of all variables to the Telomerase expression was investigated by multiple linear regression. Disease free survival was assessed by Cox's proportional hazard regression model.

Results

Telomerase positive expression was detected in 62/70 (88.6%) prostate cancer studied smears. Eight (11.4%) prostate cancer smears were entirely negative.

Telomerase expression was shown to correlate significantly with the degree of Gleason score ($p<0.001$) (Fig. 3). Telomerase expression was different in well or poorly differentiated tumours. Low Telomerase

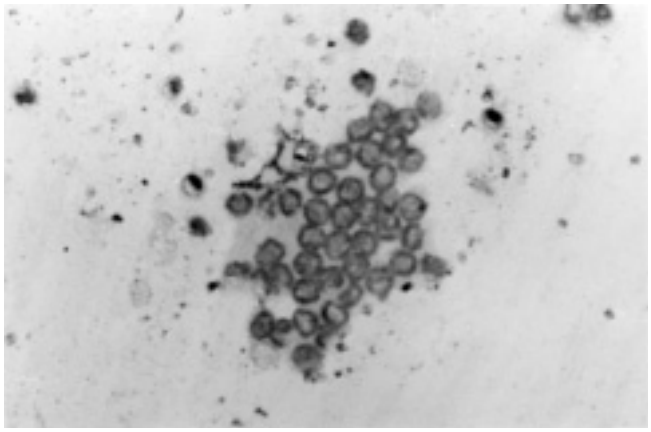

Fig. 1 - A cluster of well differentiated prostatic adenocarcinoma cells with positive nuclear expression for Telomerase (X500).

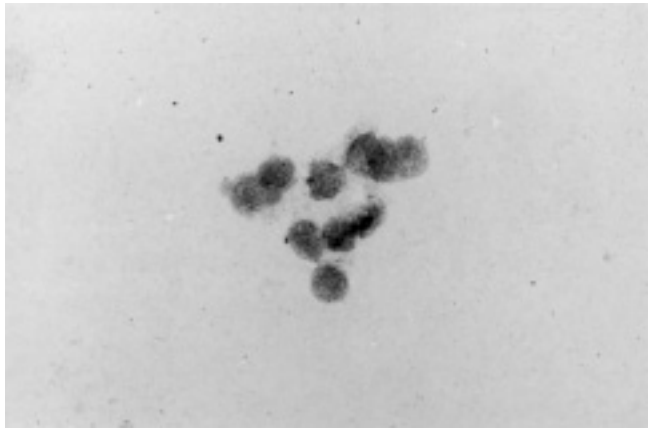

Fig. 2 - Poorly differentiated adenocarcinoma cells of prostate with positive nuclear Telomerase expression (x500)

expression was observed in well differentiated prostate cancer smears and high Telomerase expression in poorly differentiated prostate cancer smears (Fig. 4). When combining Telomerase positivity with tumour differentiation there was a significant association between these parameters ($p<0.001$).

Overexpression of Telomerase in prostate carcinoma cell smears, was associated with increase pretreatment PSA serum levels (>10) ($p=0.002$) (Fig. 5).

Figure 6 shows the distribution of Telomerase expression in prostate carcinomas according to histopathological staging. Differences were not statistically significant ($p<0.56$).

The multiple linear regression model shows that the most significant variables associated with Telomerase expression are the Gleason score ($p<0.0001$) and PSA serum levels ($p=0.0125$) (Table II).

Long rank test and Cox's Model demonstrated that Telomerase expression had significant prognostic value for the disease free survival (Table III, IV).

Discussion

Various studies on primary prostatic adenocarcinoma have suggested that approximately 47-92% of tumours demonstrate Telomerase activity (7, 8, 10, 11). This activity appears to be one of the biological markers that might be able to characterize the biological aggressiveness and the malignant nature of prostate cancer.

The current investigation demonstrated activation of Telomerase in 88.6% of the smears of surgically

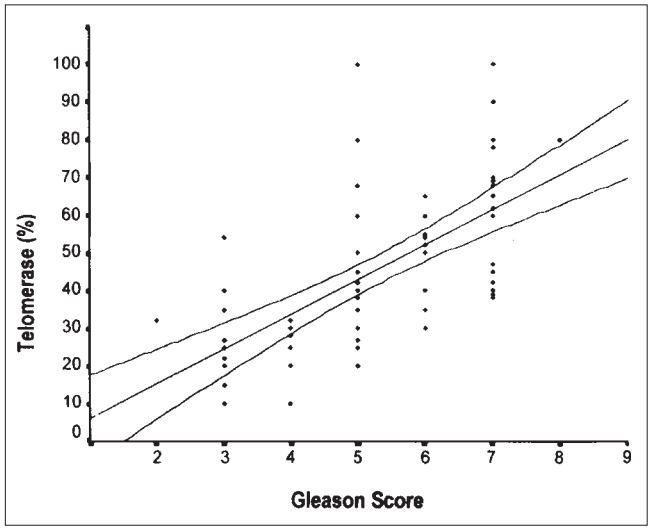

Fig. 3 - Association of Telomerase immunoreactivity with Gleason score for 70 imprint prostate adenocarcinoma smears.

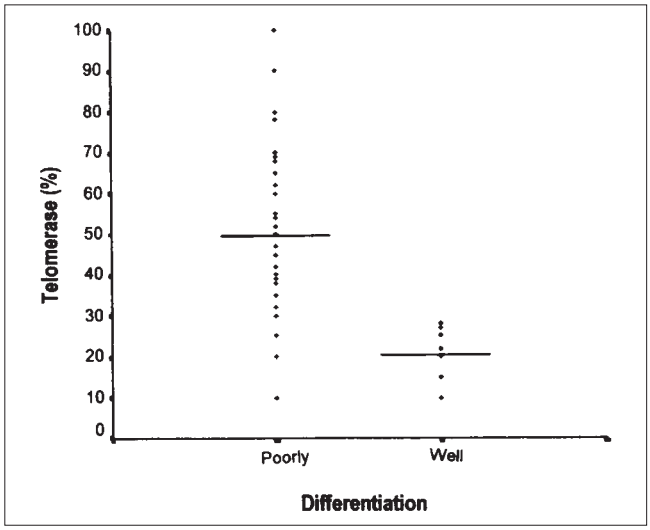

Fig. 4 - Distribution of Telomerase expression in prostate carcinomas according tumour differentiation.

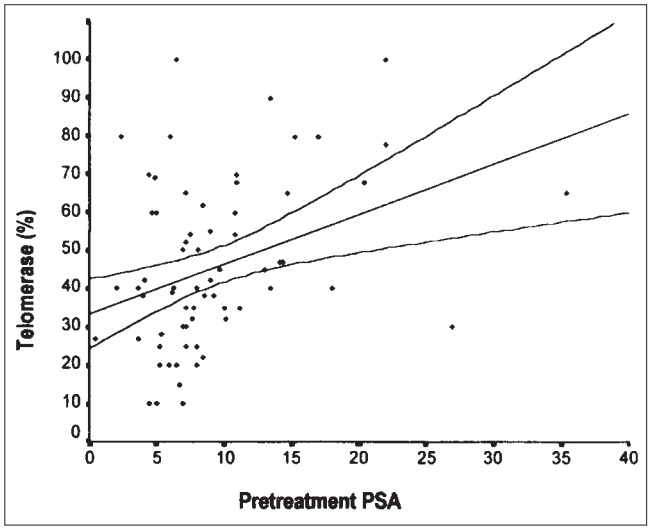

Fig. 5 - Pretreatment serum PSA concentration in relationship with Telomerase expression in smears of prostate carcinomas.

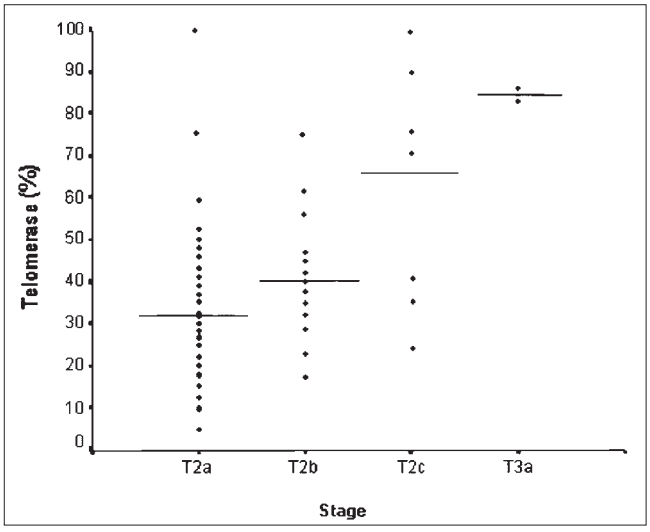

Fig. 6 - Distribution of Telomerase expression in prostate carcinomas according to histopathological staging.

resected tissues of prostate carcinoma. These findings coincide well with those of previous studies of the prostate and suggest that Telomerase activation may be crucial in the pathogenesis of prostate carcinoma (7, 11, 12).

Lack of Telomerase activity has been reported in about 10% of tumours (1,13). The negative results obtained with 11.4% of our carcinoma specimens, although histologically confirmed, is of considerable interest in view of several other reports of a strong association between Telomerase activity and malignancy. Possible explanations for the absence of this activity may be that the majority of cancer cells in these negative cases may still be mortal, the Telomerase activity may be underestimated because of a high percent of surrounding normal cells with little or no Telomerase activity and finally the presence in some smears factors may be capable of inhibiting Telomerase (11, 12, 14).

The relationship between Telomerase activity and prognosis is not yet clearly demonstrated, indeed published data concerning variably correlations between Telomerase activity and common clinical and pathological parameters are conflicting. In our study we found correlation between Telomerase expression and histopathological tumour stage, Gleason score, tumour

Table II - Contribution of various clinicopathological parameters to Telomerase expression

	Clinicopathological parameters (Dependent variable Telomerase)				95% Confidence Interval for B	
	B	Std error	t	Sign.	Lower Bound	Upper Bound
Gleason score	8.24	1.22	6.73	<0.0001	5.79	10.68
Pretreatment PSA	.82	.32	2.57	.0125	.18	1.45
Stage	.083	.945	.348			
Tumour differentiation	-0.88	-831	.409			

Table III - Assessed by multiple linear regression model (Dependent variable: Telomerase, long rank test)

	Long rank test						95% Cl for HR	
	B	SE	Wald	df	Sign.	HR	Lower	Upper
Telomerase	.148	.056	6.887	1	.009	1.159	1.038	1.294

Table IV - Cox's model Variable not in the Equation

Cox's model:Variable not in the Equation	Score	df	Sign
Stage	.057	1	.811
Gleason score	.902	1	.342
Differentiation	.000	1	.985
PSA	.258	1	.612

Residual Chi Square=13,833 with 7df Sign=.054

differentiation and preoperative PSA serum levels. High Telomerase expression was more frequently detected in poorly differentiated cancer smears compared with well differentiated and lower Gleason score cases. These results are in line with previous studies. Lin et al (7) and Meid et al (8) found that the level of Telomerase activity in prostate cancer was related significantly with Gleason score and tumour differentiation.

In the study of Wullich et al (15) no significant correlation was found between Telomerase activity and Gleason score. Similar results were observed by other investigators (7, 10, 12).

The conflicting results concerning Telomerase activity in regard to tumour differentiation and pathological grade in prostate cancer may be due to a different proportion of cancer cells per sample. In prostate cancer the infiltration of tumour cells is at 5% and Telomerase expression may be underestimated because of a large number of normal cells which are beside the malignant and expressed little or no Telomerase activity (11, 14).

Prostate specific antigen currently is the most sensitive and clinically important tumour marker available for the diagnosis and management of prostate cancer. Several large scale studies have demonstrated that serum PSA correlates well with advancing clinical stage, tumour volume and pathological stage (16, 17, 18).

In the present study the high levels of serum PSA have been shown to be associated with increased expression of Telomerase. This relation suggests the importance of this expression in determining prognosis and confirms similar results obtained by other authors (15).

Following radical prostatectomy therapy, serum PSA values decreased significantly and were below normal in 94.3% of patients within 6 to 36 months. A detectable serum PSA level (greater than 5.1 ng/ml) was the only evidence of recurrence in 5 (7.1%) cases. These results are in agreement with the Telomerase positivity staining score [80-100% (+++)] in imprints of these patients. Especially, it must be pointed out that between these patients 1, although belonging to a low stage (T2a), the Telomerase expression in the smear was 100%. Several studies have investigated the significance of a detectable serum PSA within the first year following surgery (19,20). Furthermore, according to a study of Lin et al (7) the Telomerase activity might be related to the progression of prostate cancer and is a diagnostic marker indicating malignant potential. Extremely high expression of Telomerase activity in metastatic lymph nodes was also found in other tumours (21,22).

Disease free survival was assessed by Cox's proportional hazard regression model. This analysis demonstrated that the most significant prognostic marker was the Telomerase (p=0.009).

In conclusion, our results suggested that Telomerase expression could be a marker indicating the malignant potential of prostate cancer and could be an indicator for poor prognosis in patients with prostate cancer.

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