

Research letters

Co-expression of survivin and *TERT* and risk of tumour-related death in patients with soft-tissue sarcoma

Peter Würfl, Matthias Kappler, Axel Meye, Frank Bartel, Thomas Köhler, Christine Lautenschläger, Matthias Bache, Hannelore Schmidt, Helge Taubert

Increased expression of survivin has been shown to be a negative predictor of survival in patients with soft-tissue sarcoma. We investigated 89 adults with soft-tissue sarcomas to ascertain the relation between co-expression of survivin and human telomerase reverse transcriptase (*TERT*) transcripts and prognosis. We quantified mRNA expression of survivin and *TERT* transcripts. Cox's proportional-hazards regression model showed co-expression of both genes to be a significant negative prognostic factor for patients with stage I to stage IV tumours ($p=0.0004$; relative risk 20.1, 95% CI 3.8–106.4) and for those at stage II and III ($p=0.0002$; 42.1, 6.0–294.9) compared with low expression of both genes. Co-expression of survivin and *TERT* transcripts identifies patients at high risk of tumour-related death.

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Soft-tissue sarcomas form a heterogeneous group of malignant mesodermal tumours. Prognostic factors for soft-tissue sarcomas include expression of the genes *P53*, *MDM2*, *BCL2*, and survivin.^{1,2} Besides survivin, the gene for human telomerase reverse transcriptase (*TERT*) is also expressed in almost all human cancers but not in normal tissues, with the exception of embryonic and stem cells.³ We have shown that increased expression of the survivin transcript is an

independent negative predictor of survival in patients with soft-tissue sarcoma ($p=0.009$, relative risk 2.7).² In this study, we measured levels of survivin and *TERT* transcripts in soft-tissue sarcoma samples and ascertained the relation between these levels and prognosis.

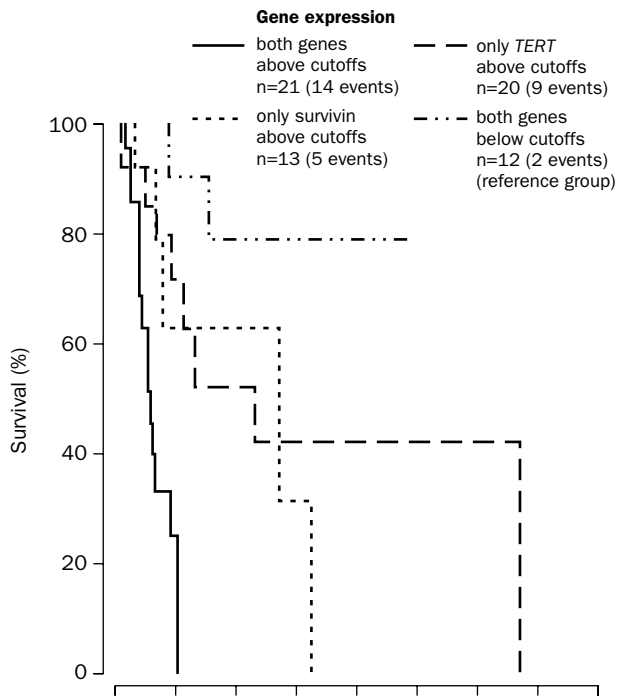
We investigated 89 adults with soft-tissue sarcoma. All patients gave written informed consent. We quantified mRNA expression of survivin by TaqMan assay (Roboscreen GmbH, Leipzig, Germany) and *TERT* transcript expression by LightCycler assay (TeloTAGGG hTERT Quantification Kit, Roche, Mannheim, Germany).² High-level expression of survivin was defined as a relative value greater than 2.0×10^{-3} of the survivin expression value standardised to GAPDH levels. High-level expression of *TERT* was defined as a relative value greater than 0.8 ($\times 10^{-3}$) of the *TERT* expression value standardised to the expression value of the porphobilinogen deaminase gene. Both these levels of expression are above the mean expression levels in normal tissues adjacent to tumour tissues.

We did multivariate analysis according to Cox's proportional hazards regression model (with adjustment for staging, tumour entity, tumour localisation, and type of tumour resection) to estimate the effect of transcript detection of survivin and *TERT* as prognostic factors. We also did

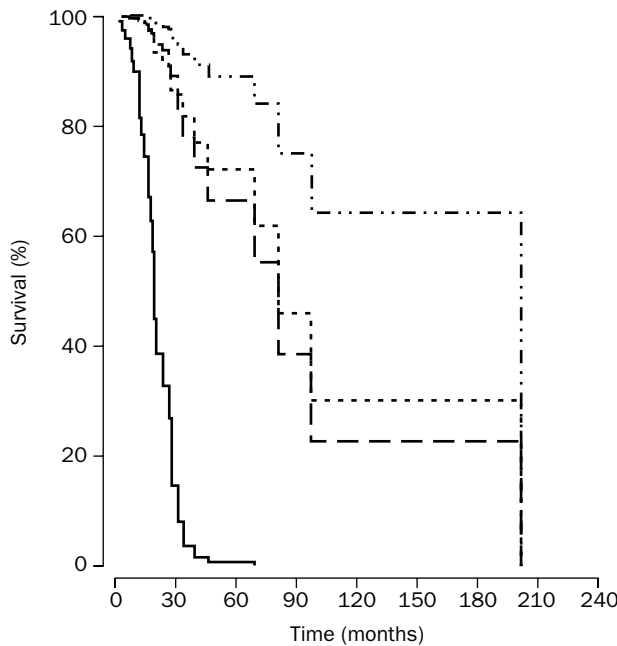
	Total (n=89)	Low <i>TERT</i> and low survivin (n=19)	High <i>TERT</i> and low survivin (n=26)	Low <i>TERT</i> and high survivin (n=18)	High <i>TERT</i> and high survivin (n=26)	Patients at follow-up	
						Alive* (n=51)	Dead† (n=38)
Men/women	42/47	9/10	12/14	8/10	13/13	24/27	18/20
Tumour type							
Primary tumours	59	12	16	11	20	35	24
Recurrences	24	7	7	6	4	14	10
Metastases	6	0	3	1	2	2	4
Histological subtype							
Liposarcoma	21	7	7	4	3	15	6
MFH/fibrosarcoma	28	7	6	8	7	18	10
NS	13	1	6	0	6	6	7
RMS and LMS	17	2	4	5	6	7	10
Other STS	10	2	3	1	4	5	5
Tumour stage							
I	14	6	2	5	1	14	0
II	38	8	13	7	10	26	12
III	28	4	7	6	11	10	18
IV	9	1	4	0	4	1	8
Tumour localisation							
Extremities	56	14	16	13	13	40	16
Thorax	6	1	2	1	2	2	4
Head	4	1	1	0	2	1	3
Abdomen	23	3	7	4	9	8	15
Tumour resection grade							
Radical (R0)	58	16	15	10	17	39	..
Not radical (R1)	31	3	11	8	9	12	..
Follow-up stage I–IV							
Alive*	51	17	13	13	8	..	19
Dead†	38	2	13	5	18	..	19

Data are number of patients. MFH=malignant fibrous histiocytoma; NS=neurogenic sarcoma; LMS=leiomyosarcoma; RMS=rhabdomyosarcoma, STS=soft-tissue sarcoma. *After an average observation time of 35.4 months; †patients died after an average 25.2 months.

Histopathological and clinical data



Patients at risk		0-30	30-60	60-90	90-120	120-150	150-180	180-210	210-240
N	66	20	11	5	2	1	1	0	0
Events	0	23	26	28	29	29	29	30	30
Censored	0	23	29	33	35	36	36	36	36



Patients at risk		0-30	30-60	60-90	90-120	120-150	150-180	180-210	210-240
N	66	20	11	5	2	1	1	0	0
Events	0	23	26	28	29	29	29	30	30
Censored	0	23	29	33	35	36	36	36	36

Kaplan Meier analysis (upper) and Cox's proportional-hazards regression model* (lower)

*Adjusted for tumour entity, type of tumour resection, tumour localisation, and tumour stage for 66 stage II and stage III patients with soft-tissue sarcoma in 240 months. Cutoffs for expression of survivin and *TERT* were $>2 \times 10^{-3}$ survivin standardised to *GAPDH* and $>0.8 (\times 10^{-3})$ of the *TERT* expression value standardised to the expression of the porphobilinogen deaminase gene.

Kaplan-Meier analysis that excluded patients with stage I and IV tumours. We did this analysis because survival of these patients might not be decided by expression of survivin and *TERT*.

Expression levels of survivin and *TERT* are shown in the table. The cumulative 2-year survival rate was 27.9% for patients with increased expression of survivin and *TERT* compared with 100% for patients with no detectable survivin and *TERT* transcripts ($p < 0.0001$).

Cox's proportional-hazards regression model for stage I to stage IV patients showed co-expression of both genes to be a significant negative prognostic factor ($p = 0.0004$; relative risk 20.1, 95% CI 3.8–106.4) compared with low expression of both genes. High-level expression of *TERT* alone was still associated with an increased risk of tumour-related death ($p = 0.034$; 5.8, 1.1–29.6) whereas the risk with survivin alone was not statistically significant ($p = 0.097$; 4.4, 0.8–25.0).

No patients with stage I tumours died, and patients with stage IV tumours had a poor outlook due to presence of metastases. In a Cox's proportional-hazards regression model (figure), patients with stage II and III cancer, who co-expressed survivin and *TERT*, had the worst outlook compared with those who had no expression of either transcripts ($p = 0.0002$; 42.1, 6.0–294.9). In patients with stage II and III tumours, high-level expression of only one gene—ie, either survivin ($p = 0.26$; 2.7, 0.5–14.9) or *TERT* ($p = 0.15$; 3.3, 0.7–16.8) was not significant.

Survivin belongs to the family of genes which inhibit apoptosis, and *TERT* accounts for the immortal status of cells. Both genes control major steps in cancer development—ie, indefinite tumour-cell growth and cell division. Most genes which inhibit apoptosis, and *TERT*, are located close to the telomeres in different species, suggesting that maintenance of telomeres might be connected with correct functioning of survivin and *TERT*.

Stage II and stage III tumours could have a similar histomorphological appearance, but knowledge of the expression of *TERT* and survivin could help predict behaviour and outlook for patients. The level of transcription and protein expression of survivin in patients with neuroblastoma and several subtypes of soft-tissue sarcoma is correlated with low survival rates, unfavourable prognoses, and accelerated recurrences.⁴ Independent of this fact, the catalytic protein of the telomerase ribonucleoprotein complex, *TERT*, which is the critical component of telomerase activity, can participate in progression of specific histological variants of liposarcoma.⁵ Altogether, telomerase activity might be associated with increasing tumour progression in soft-tissue sarcoma.³ Why do increased survivin and *TERT* transcript levels have such an important effect on prognosis for soft-tissue sarcoma—ie, a 42-fold increased rate of tumour-related death? A possible explanation could be that soft-tissue sarcoma might originate from mesodermal stem cells and not from differentiated adult cells.

Co-expression of survivin and *TERT* transcripts correlates with very poor outlook for patients with soft-tissue sarcoma. These genes define the highest risk for tumour-related death and might have some potential as a predictor of survival.

Contributors

P Würll was responsible for genetic analysis, tissue collection, data collection, statistical analysis, and writing of the report. M Kappler was responsible for genetic analysis, statistical analysis, and writing of the report. A Meye was responsible for genetic analysis and writing of the report. F Bartel did statistical analysis and wrote the report. T Köhler did genetic analysis. C Lautenschläger did statistical analysis. M Bache was responsible for genetic analysis, tissue collection and data collection. H Schmidt was responsible for tissue and data collection. H Taubert was responsible for tissue collection, data collection, statistical analysis, and writing of the report. All authors approved the final report.

Conflict of interest statement

None declared.

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One-step immunochromatographic assay for screening of coeliac disease

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Tissue transglutaminase is the autoantigen that elicits endomysial antibodies, which are the serological hallmarks of coeliac disease. We describe a simple, rapid immunochromatographic assay for IgA and IgG antibodies to transglutaminase, which is highly accurate for diagnosis of this disease. Results were positive for all samples from 50 untreated coeliac patients, and negative for 40 non-coeliac patients with gastrointestinal disorders. The assay seems to be a useful alternative to biopsy for mass screening for coeliac disease.

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Coeliac disease is one of the most common food-sensitive enteropathies in man. Although the definitive diagnosis of coeliac disease is based on characteristic histological changes seen in jejunal biopsy specimens, serological tests, such as the detection of circulating antibodies to gliadin and to endomysium, are cheaper, less invasive methods of screening for this disease.

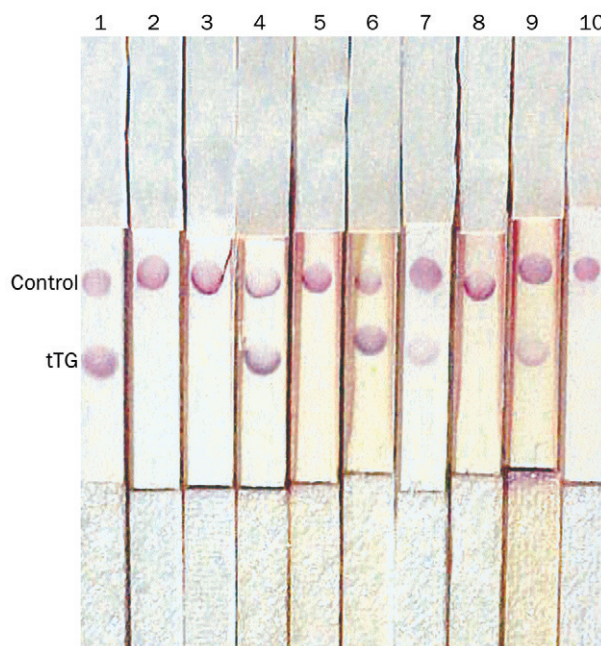
Since 1997, when Dietrich and co-workers identified tissue transglutaminase (tTG) as the major antigen recognised by antibodies to endomysium,¹ different ELISAs have been developed for the detection of autoantibodies to tTG as predictors of coeliac disease.² Balders and colleagues reported a rapid dot blot assay for the detection of antibodies to transglutaminase.³ We describe a one-step immuno-

chromatographic assay for the detection of both IgA and IgG antibodies to transglutaminase in human serum or plasma.

1 µL of guineapig tTG (Sigma, T-5398, lot 99H7425), at 1.5 g/L in 50 mmol/L Tris buffered saline with 5 mmol/L CaCl₂, pH 7.5, was adsorbed onto one end of a plastic-backed nitrocellulose membrane strip (pore size 10 µm) to form a reactive zone. A polycationic substance that binds tTG-colloidal gold conjugates was adsorbed onto the same membrane to form a control zone adjacent to the reactive zone. tTG was conjugated to colloidal gold particles and dried onto an inert fibrous support (type CNPF-S1-L2-P50, Advances Microdevices PVT Ltd, Ambala Cannt, 133001, India) which was attached to the plastic backing of the nitrocellulose strip, to achieve minimum direct contact with the beginning of the nitrocellulose membrane, close to the reactive zone. In this system, when the conjugate support is dipped in serum or plasma, any antibodies to tTG in the sample react with the colloidal-gold tTG, developing an immunocomplex that migrates through the membrane strip. The immobilised tTG in the nitrocellulose reacts with the immunocomplexes, forming a coloured dot in the reactive zone. Excess of conjugate and immunocomplexes continue migration and finally react with the control reagent, forming a second coloured dot on the strip. A positive result, indicating the presence of antibodies to tTG in the sample, is seen as two dots on the strip, with a pink-to-purple color. A negative assay shows only a control dot (figure). Results are obtained in less than 10 minutes.

Serum or plasma samples were obtained from patients and controls after informed consent. Two independent observers who were unaware of the patients' diagnosis made the test readings. There was full agreement between the two.

The immunochromatographic assay results were positive for all samples from 50 untreated patients with coeliac disease diagnosed on the basis of the revised European Society of Paediatric Gastroenterology And Nutrition criteria for coeliac disease,⁴ for a sensitivity of 100% (95% CI, 92.9–100%). The same samples were tested for IgA antibodies to transglutaminase with a commercial ELISA (Celikey,



Detection of antibodies to tissue transglutaminase by immunochromatographic assay

1, 4, 6, 7, and 9: positive samples; 2, 3, 5, 8, and 10: negative samples.