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The influence of reactivation of the telomerase in tumour tissue on the prognosis of squamous cell carcinomas in the head and neck

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BACKGROUND: The reactivation of the telomerase seems to be an important step in the carcinogenesis of most human cancer types. Cell clones, which express this enzyme, get the ability of indefinite proliferation, means become immortal.

METHODS: In this study, 80 patients with squamous cell carcinomas (SSC) in oral cavity, oropharynx, hypopharynx and larynx were recorded prospectively concerning a possible correlation of telomerase activity and clinical and prognostic factors. Telomerase activity was analysed by a modified telomeric repeat amplification protocol (TRAP) assay.

RESULTS: In 75% of the tumour tissues the telomerase was demonstrated independently of the localization of the tumour. The known clinical prognostic factors did not show any correlation to the expression rate of the telomerase activity in the tumour tissues. Also, reactivated telomerase did not affect the tumour-dependent survival. Only the number of lymph node metastases was in tendency higher in patients with telomerase-positive tumours. The number and timeframe of local and regional recurrences was not influenced by the telomerase status.

CONCLUSIONS: Although telomerase seems to be an important part of the carcinogenesis of SCC our data show that the reactivation of telomerase in tumour tissue did not have any prognostic significance for these tumours. The tendency that tumours with active telomerase developed lymph node metastases in a higher number should be evaluated by further enlarged studies for its clinical relevance.

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Introduction

Tumour cells are characterized by increased mitotic activity. Because of mutations in tumour suppressor genes or proto-oncogenes, they are able to bypass their natural physiological senescence. According to the model of replicative senescence the cell population reach finally a limitative step of proliferation because of the progressive shortening of the telomeres (1, 2). The telomeres are an important component of the regulation of cellular senescence as they may function-like a mitotic clock, which regulates s the lifespan of cells. If telomeres reach a point of critical length telomerase might be reactivated in some cells and in consequence stop the process of replicative senescence. Thus, cells that can express the enzyme telomerase may escape from cellular senescence and possibly gain the ability of indefinite proliferation (for review see 2, 3). There is no telomerase activity in most normal somatic cells. But telomerase is active in a few cell types, which are characterized by unlimited cell divisions (e.g. germ cells and somatic stem cells). Also the telomerase activity is observed in activated lymphocytes in a low quantity (4).

The present knowledge in the literature shows that reactivation of telomerase is an important molecular biological change in the carcinogenesis of head and neck cancer (5–9). As this enzyme was found to be active in most SCCs investigated it was suggested that the telomerase status in the tumour could be a helpful diagnostic and prognostic marker. But the importance of the reactivation for the clinical prognosis of the patients and the correlation to the clinical follow-up is not investigated up to now. Thus, we screened 80 patients with SCC of the oral cavity, oropharynx, hypopharynx and larynx whether there exists a correlation between the telomerase status of their tumours and

the well-established prognostic factors for head and neck cancer, tumour localization, survival rate and occurrence of recurrences.

Material and methods

Tumour samples

From 01.09.1995 to 31.12.1997, 80 patients were recorded for this study prospectively. All patients were treated through operative therapy of their tumour with curative aim. There were recorded only squamous cell carcinomas (SCC) in oral cavity, oropharynx, hypopharynx and larynx, which were operable. All patients were free from distant metastases and synchronous secondary primary tumours. Tumour specimens were staged according to the tumour nodes metastasis (TNM) classification system of malignant tumours. Informed consent was obtained from all patients for the use of tissue samples in research.

The tumour samples and matched normal mucosa were obtained after surgical resection; they had been collected fresh, frozen in liquid nitrogen and were stored at −80°C for further investigations.

Telomerase assay (telomeric repeat amplification protocol)

For telomeric repeat amplification protocol (TRAP) assay the S100 protein extract was isolated from approximately 50 mg of the homogenized frozen normal and tumour tissue as described by Kim et al. (10). The further procedure was performed as described by Fiedler et al. (11). In brief, the concentration of protein was measured using the Bio-Rad protein assay kit (Bio-Rad, Munich, Germany). Then, an aliquot of the extract representing 1.5 μg of protein solution was mixed with 1X reaction buffer (Eurogentec, Seraing, Belgium), 1.5 mM $MgCl_2$, 50 μM dNTPs, 0.05 μg TS oligonucleotide (5′-AATCCGTCGAGCAGA GTT-3′, labelled with infrared dye IRD800; Boehringer Mannheim, Mannheim, Germany) and 0.5 μg T4 gene 32 protein (Pharmacia, Freiburg, Germany) in a total volume of 25 μl. The reaction mixture was incubated for 10 min at 23°C for telomerase-mediated extension of the TS primers and then heated at 94°C for 90 s for inactivating the telomerase. After adding 15 μg of an internal polymerase chain reaction (PCR) standard-DNA a, 0.05 μg CX primer (5′-CCTTACCCTTACCCT-TACCCTTA-3′) and 1 unit of *Taq* DNA polymerase (Eurogentec) the probes were subjected to 31 PCR cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 45 s. Finally, 2 μl of each PCR product was separated by electrophoresis on a 6% denaturing polyacrylamide gel using a LI-COR DNA sequencer (LI-COR, Lincoln, NB, USA). The laser detection system of the LI-COR visualizes the extended and amplified PCR products as an increasing DNA ladder. If a protein lysate displayed a negative activity in TRAP, the analysis was repeated by mixing it with a known positive lysate to exclude inhibitory factors.

Results

The investigation of telomerase activity was performed in 80 tumour tissues from head and neck cancers from different localizations within the upper aerodigestive tract. Table 1 demonstrated the correlation between the localization and the clinical stage (UICC) or stadium of the investigated tumours.

Telomerase activity was detectable in 75% of the investigated tumour tissues. The presence and absence of telomerase activity in correlation to the localization of the tumour is presented in Table 2. Oropharyngeal (75%) and laryngeal cancers (69%) showed telomerase activity in less cases than hypopharyngeal cancers (82%) and carcinomas of the oral cavity (82%). The differences proved to be not significant (chi-square test, Likelihood quotient). The reactivation of telomerase did not show correlations to the known clinical prognostic factors-like TNM stadium, tumour stadium, patho-histological grading and differentiation.

There was no telomerase activity in the parallel controls of normal clinically inconspicuous mucosa resected from areas in wide distance to the tumour area.

The tumour-dependent survival of the patients was not influenced by the telomerase status of the tumour tissues (Fig. 1). The differences did not show any significance in log-rank test.

Because of the prognostic relevance of lymphatic spread in head and neck cancer we investigated the correlation between the telomerase activity and the lymphatic spread of the tumour at the time of first diagnosis (Table 3). Tumours with telomerase developed lymph node metastases in a higher rate than tumours without telomerase (58% vs. 45%) but the difference was not significant.

Table 1 Telomerase active tumour tissues in relation to localization and stadium of the investigated tumours

	Tumour stadium				
Localization	*I*	*II*	*III*	*IV*	*Total*
Oral cavity	2	4	1	4	11
Oropharynx	0	4	2	26	32
Larynx	4	5	6	11	26
Hypopharynx	0	0	2	9	11
Total	6	13	11	50	80

Table 2 Telomerase status in the tumour tissues in relation to the tumour localization

	Telomerase		
Localization	*No*	*Yes*	*Total*
Oral cavity	2	9	11
Oropharynx	8	24	32
Larynx	8	18	26
Hypopharynx	2	9	11
Total	20	60	80

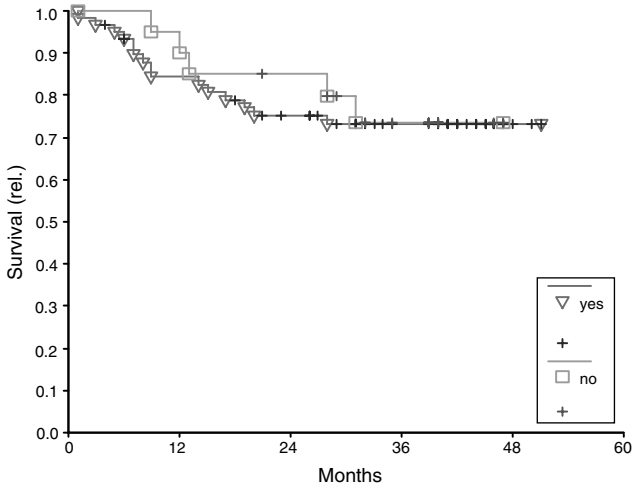

Figure 1 Tumour-dependent survival in relation to the telomerase activity in tumour tissues (yes = telomerase activity, no = none).

Table 3 Telomerase activity in the primary tumour in relation to the lymph node metastases

	Lymph node metastases		
Telomerase	*No (N0)*	*Yes (pN+)*	*Total*
No	11	9	20
Yes	25	35	60
Total	36	44	80

Table 4 Telomerase activity in the primary tumour in relation to the number of local recurrences

	Local recurrences			
Telomerase	*0*	*1*	*2*	*Total*
No	16	3	1	20
Yes	49	11	0	60
Total	65	14	1	80

Generally, the presence of local recurrences is a helpful factor in the assessment of further clinical course and impaires the prognosis. Thus, we correlated telomerase activity in tumour tissue and the occurrence of local recurrences as it is demonstrated in Table 4. The rate of recurrences with and without telomerase (18% vs. 20%) was nearly the same. The reactivation of the telomerase in the tumour has no influence on the developing of a local recurrence and also the timeframe for developing a local recurrence was not influenced by the reactivation of the telomerase (Fig. 2).

Beside local recurrences also the appearance of regional recurrences in the lymph nodes could affect the prognosis for the head and neck cancer patient. About 18% (11 of 60) of the patients with detectable telomerase in the tumour tissue and nearly the same number (three of 20 patients, 15%) of patients without telomerase developed regional recurrences. The time for developing the regional recurrence shows no correlation to the telomerase activity in tumour tissue.

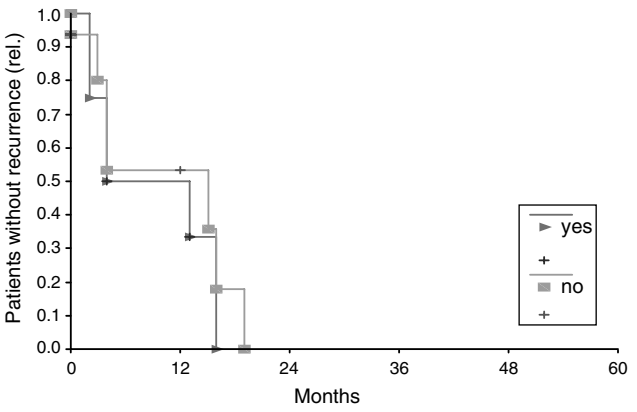

Figure 2 Time to the local recurrences in dependence to the presence of telomerase activity in tumour tissues (yes = telomerase activity, no = none).

Discussion

Potential tumour cells have to bypass the problem of progressive shortening of the telomeres caused by the increased proliferation rate. In the known models of carcinogenesis only some cell clones of the heterogeneous tumour cell population are able to do this. These cells reactivated the enzyme telomerase, which stabilize the length of the telomeres (12).

The aim of our study was the investigation of 80 tumour tissues of head and neck cancers from different localization of the upper aerodigestive tract with a modified TRAP assay (10, 11) and the correlation of the results to the known prognostic factors in a clinical follow-up.

The data in the literature give the opinion that the reactivation of the telomerase is a fact in the carcinogenesis of many different tumour types including tumours of the head and neck. Our results of the detection of a telomerase activity in squamous cell carcinomas of the head and neck (HNSCC) are comparable with the data in the literature (5–14).

Up to now investigations of a correlation of the telomerase activity in different tumour localization with the data of clinical follow-up for tumours of the upper aerodigestive tract are not published. Most of the studies reported rates of telomerase activity in different tumour localizations and a possible correlation of known prognostic factors but a correlation of the clinical follow-up with the survival rates was not performed. In our cohort of analysed tumours it can be noticed that the reactivation of the telomerase plays an important role in the carcinogenesis of these tumours. In 75% of the investigated tumours telomerase activity could be proved. Most tumours with telomerase reactivation were carcinomas of the oral cavity and the hypopharynx whereas in carcinomas of the oropharynx and larynx we could demonstrate a lower appearance of the telomerase. The differences between the localizations were not significant. Mao et al. (5) found telomerase activity in 90% of the examined carcinomas of oral cavity just like Sumida et al. (13). Curran et al. (14)

showed telomerase activity in 85% of the laryngeal cancers.

Like Miyoshi et al. (9) in our investigations telomerase activity was not detectable in the normal control specimens. Several other authors reported of telomerase-positive normal tissues, e.g. Loughran et al. (8) displayed telomerase activity in 74% of the tumour-free tissue. But nothing is described about the distance between the tumour and the localization of the control tissue and the patho-histological investigation of the tumor-free state of the used control tissue. To address this in our study, the control tissues were resected in a far distance from the tumour area and pathological evaluation for the absence of tumour cells was performed in each case.

Our data show clearly for the first time that telomerase activity in tumour tissue of the head and neck did not have any influence on the tumour-dependent survival. For tumours outside the upper aerodigestive tract, Ferlicot et al. (15) described a correlation of the telomerase activity with the prognosis in hepatocellular cancer. Similar was reported by Carey et al. (16) and Bieche et al. (17) for breast cancer, Hiyama et al. (18) for oesophageal cancer and Halvorsen et al. (19) for prostatic cancer. Fujitota et al. (20) did not find any influence of the telomerase on tumour-dependent survival in patients with renal cell carcinoma.

It seems interesting to us that patients with active telomerase in the tumour at the time of first diagnosis developed in tendency more frequently lymphatic spreads. Lymph node metastases were found in 58% of the telomerase-positive tumours but only in 45% of the telomerase-negative tumours. Sumida et al. (21) could not find a connection between telomerase activity and the extent of lymphatic spread in oral malignancy. Hyiama et al. (22) described a correlation of the telomerase activity with the extent of the lymphatic spread for gastric cancer and Onoda (23) found the same for thyroid gland cancer.

The frequency of occurrence of local recurrences seems to be independent of the telomerase status of the primary tumour in our investigated patients. During the follow-up patients with telomerase-positive tumours developed in 18% a local recurrence in contrasts to 20% local recurrences in telomerase-negative tumours. To our best knowledge these data are the first published report of investigation of telomerase activity and local recurrences in SCC of the upper aerodigestive tract in a large cohort of patients. Only in cervix carcinoma a correlation between the number of regional recurrences in cervical lymph nodes and the appearance of the telomerase in tumour tissue was investigated. Here, tumours with telomerase activity developed regional recurrences in the same rate-like tumours without telomerase (18% against 15%), too.

Furthermore, our study show that the reactivation of telomerase in tumour tissue had no effect on the timeframe of developing a regional recurrence. Again, data of a correlation between number of regional recurrences and the time of recurrence to the reactivation of the telomerase are not published yet by other.

In conclusion, the reactivation of telomerase is an important part of the carcinogenesis of SCC of the pharynx and larynx. Our data show clearly that the reactivation of telomerase in tumour tissue have no prognostic significance for these tumours in our cohort of 80 investigated patients. The observed tendency that patient with active telomerase in tumour tissue developed in a higher number lymph node metastases could have prognostic clinical relevance but must be confirmed in further studies.

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