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Angaben zur Veröffentlichung / Publication details:

Fenner, Matthias, Falk Wehrhan, Marc Jehle, Kerstin Amann, Martin Radespiel–Tröger, Gerhard Grabenbauer, Johannes Zenk, Emeka Nkenke, Martin Schinhammer, and Stefan Schultze–Mosgau. 2005. "Restricted-expressed proliferation–associated protein (Repp86) expression in squamous cell carcinoma of the oral cavity." *Strahlentherapie und Onkologie* 181 (12): 755–61. https://doi.org/10.1007/s00066-005-1430-7.

Restricted-Expressed Proliferation-Associated Protein (Repp86) Expression in Squamous Cell Carcinoma of the Oral Cavity

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Purpose: To determine the expression of repp86 (restricted-expressed protein of 86 kDa theoretical molecular mass), a proliferation-associated protein expressed in S-, G_2- and M-phases of the cell cycle, in samples of normal mucosa as well as squamous cell carcinoma of the oral cavity (OSCC).
Patients and Methods: The repp86 labeling index (LI) was determined imunohistochemically in ten samples of normal oral mucosa and 59 samples of OSCC. Repp86 LI was correlated with tumor stage, histopathologic grading, and the expression of Ki-67 and topoisomerase IIα.
Results: Repp86 was detectable in all tissues analyzed. The mean LI was 4.7% for normal mucosa and 18.4% for squamous cell carcinoma ($p < 0.0001$). Repp86 expression was not related to tumor size, lymph node invasion, or histopathologic grading but was positively correlated with Ki-67 index ($r = 0.48$; $p < 0.01$) as well as with topoisomerase IIα ($r = 0.39$; $p < 0.01$). Ki-67 and topoisomerase IIα levels were also significantly correlated with each other ($r = 0.34$; $p < 0.05$).
Conclusion: These results indicate that repp86 expression can be an additional proliferation marker among Ki-67 and topoisomerase IIα in OSCC. Further research will be directed at the evaluation of the prognostic value of repp86 expression in OSCC as well as in leukoplakia and early-stage OSCC.

Repp86-Expression beim Plattenepithelkarzinom der Mundhöhle

Ziel: Erfassung der Expression von Repp86 („restricted-expressed proliferation-associated protein"), eines in den Zyklusphasen S, G2 und M exprimierten, proliferationsassoziierten Proteins, bei Normalschleimhaut und Plattenepithelkarzinomen der Mundhöhle.
Patienten und Methodik: Im Rahmen dieser retrospektiven Untersuchung wurde formalinfixiertes histopathologisches Routinematerial von 59 Patienten mit Plattenepithelkarzinomen der Mundhöhle sowie zehn Proben unveränderter normaler Mundschleimhaut untersucht. Die Markierungsindizes von Repp86, Ki-67 und Topoisomerase IIα wurden immunhistochemisch ermittelt und mit klinischen Parametern korreliert.
Ergebnisse: Repp86 konnte in allen untersuchten Gewebeproben nachgewiesen werden. Der mittlere Markierungsindex betrug 4,7% in normaler Mundschleimhaut sowie 18,4% in Proben von Plattenepithelkarzinomen der Mundhöhle ($p < 0,0001$). Ein signifikanter Zusammenhang zwischen Repp86-Expression und TNM-Stadium bzw. histopathologischem Differenzierungsgrad zeigte sich nicht. Dagegen korrelierten die Markierungsindizes von Repp86 und Ki-67 bzw. Topoisomerase IIα ($r = 0,48$; $p < 0,01$ bzw. $r = 0,39$; $p < 0,01$) sowie die Markierungsindizes von Ki-67 und Topoisomerase IIα ($r = 0,34$; $p < 0,05$).
Schlussfolgerung: Die Ergebnisse dieser Studie zeigen, dass die Repp86-Expression zur Beurteilung der Proliferationsaktivität beim Plattenepithelkarzinom der Mundhöhle herangezogen werden kann. Im Rahmen weiterer Studien wird die prognostische Bedeutung der Repp86-Expression beim Plattenepithelkarzinom der Mundhöhle untersucht werden.

Schlüsselwörter: Plattenepithelkarzinom der Mundhöhle · Repp86 · Proliferation

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Introduction

Squamous cell carcinomas of the oral cavity (OSCC) constitute a major proportion of head-and-neck cancers with 10,846 new cases diagnosed in the Federal Republic of Germany in 1998 [9, 17, 21].

The prognostic indicators currently in common use are tumor size, lymph node invasion, and histopathologic grading [9, 10]. Immunohistochemical proliferation indices, although reported to be of relevance, have not become established prognostic indicators yet. In contrast to other tumor entities, data on the expression of proliferation markers have yielded ambiguous results in OSCC [3, 7, 8]. The most common immunohistochemical marker used to study cell proliferation is the Ki-67 antigen, which describes the global growth fraction, defined as the cell population in G_1-, S-, G_2-, and M-phases of the cell cycle [6]. The inevitable inclusion of G_1-phase cells with uncertain destinies might account for the limited utility of proliferation markers such as Ki-67 and topoisomerase IIα[14, 15, 19, 31]. The G_1-phase cells may resume cycling but may remain in G_1 for an indeterminate period of time, or they may definitely leave the cell cycle to become quiescent or succumb by apoptosis. However, once cells have crossed the restriction point and become engaged in DNA replication, they are determined to complete a division cycle because cell cycle progression depends on an intrinsic program that is largely refractory to external influences [13, 19].

In 1997, Heidebrecht et al. reported a hitherto unknown proliferation-associated protein, which later was defined as repp86 (i.e., restrictedly expressed proliferation-associated protein) [13].

In mammals, repp86 expression was shown to be cell cycle-regulated, with a diffuse nuclear distribution becoming apparent at the onset of S-phase, persisting through G_2-phase, relocating to the mitotic spindle in M-phase, and vanishing with the completion of cytokinesis [19]. Due to cloning of the cDNA, repp86 has recently been characterized as the human homologue of the *Xenopus* protein TPX2 [12, 34]. This molecule is responsible for the chromatin-induced microtubule formation during mitosis and is able to influence the rate of mitosis quantitatively [11]. In immunohistochemical analyses of normal tissues and different tumor entities, the labeling index (LI) was only about 40% of the Ki-67-positive cell fraction. As repp86 is expressed exclusively in cell cycle phases S, G_2, and M, it reflects the biologically relevant fraction of proliferating cells more accurately [17, 19, 22, 24].

In this study, we evaluate the expression of repp86 in normal oral mucosa as well as in samples of OSCC. The expression is analyzed quantitatively in relation to tumor stage, histopathologic grading as well as to the expression of Ki-67 and topoisomerase IIα.

Patients and Methods

Tissue Samples

After approval by the Ethics Committee of Friedrich Alexander University Erlangen-Nuremberg, Germany (ethics approval 2430), tissue samples were obtained from ten healthy subjects during routine elective surgical procedures. The samples were taken from the floor of the mouth in three cases and from the buccal plane in seven cases. The specimens were fixed in 10% buffered formalin and embedded in paraffin. All subjects had signed informed consent a minimum 24 h before surgery.

Paraffin-embedded tumor samples of patients with OSCC were retrieved from the Department of Pathology of Friedrich Alexander University Erlangen-Nuremberg. For statistical analysis, age, sex, tumor size, regional lymph node metastasis, and histopathologic grading according to UICC criteria were documented by the Cancer Center of Friedrich Alexander University Erlangen-Nuremberg.

Patient selection was performed according to an in-/exclusion protocol. The inclusion criteria were: primary OSCC diagnosed between January 1, 1996 and December 31, 1999, therapy with a curative intent, tumor resection (R0), and pre- or postoperative radiotherapy/radiochemotherapy. Patients who had a history of other malignancies, immune disorders or immunosuppression were excluded. We retrieved 185 patients who met the in- and exclusion criteria. Of these, 59 patients were selected randomly for further analysis.

The cohort of tumor patients included 51 male and eight female patients. Patient age at diagnosis ranged from 35 to 73 years, the average was 56 years. Four tumors (7%) were grade 1, 38 tumors (64%) grade 2, and twelve tumors (21%) grade 3. Information was missing for five tumors (8%). According to the TNM classification, three tumors (5%) were characterized as T1, 24 tumors (41%) were T2, three tumors (5%) T3, 27 tumors T4 (46%), and information was missing for two tumors (3%). In 20 patients (34%) a lymph node invasion could not be detected, 13 patients (22%) were diagnosed N1, and 22 patients were diagnosed N2 (37%). Information on lymph node invasion was missing in four patients (7%).

Immunohistochemical Analyses

Expression of the mitotic spindle-associated protein repp86 was analyzed immunohistochemically with the monoclonal antibody Ki-S2. Briefly, 3–5 µm thick sections were cut from formalin-fixed, paraffin-embedded tumor specimens, mounted on sialanized slides, and dried overnight at 37 °C. Immunoreactivity was restored by incubation of the slides with target retrieval solution at 100 °C in a water bath for 30 min (S1700, DAKO, Glostrup, Denmark). To prevent nonspecific binding of the secondary antibody and the ABComplex with the tissue section, the slides were subsequently incubated with avidin and biotin solutions for 15 min each (X0590, DAKO) as well as with rabbit serum for 30 min (X0902, DAKO).

For qualitative and quantitative analysis of repp86 expression, the avidin-biotin peroxidase complex (ABC-POX) method was used. Repp86 was detected by incubation of the slides with the undiluted monoclonal mouse-IgG antibody Ki-S2 (dilution 1 : 5 in TBS, Department of Pathology and

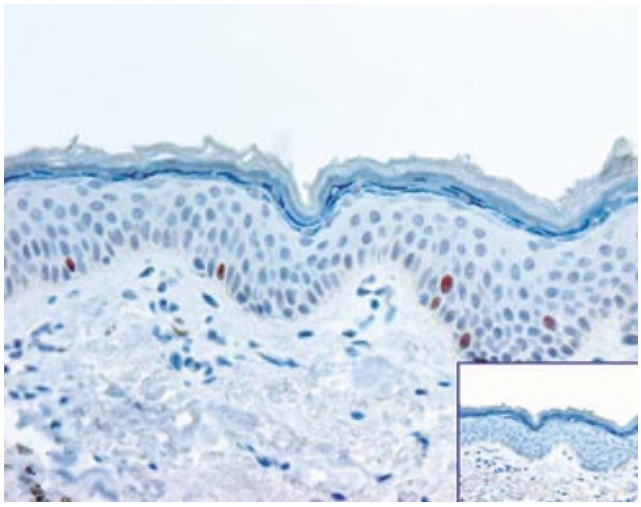

Figure 1a – Abbildung 1a

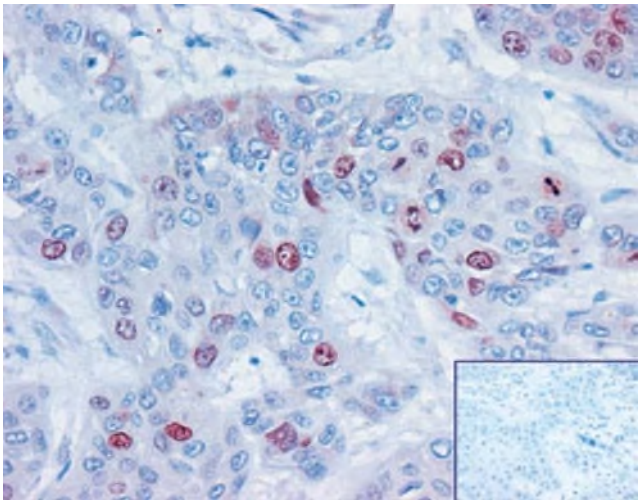

Figure 1b – Abbildung 1b

Figures 1a to 1c. a) Normal mucosa of the oral cavity with staining for repp86 and negative control. Staining procedure: streptavidin-biotin-peroxidase technique, hemalum counterstain, original magnification ×400. b) OSCC with staining for repp86 and negative control. Staining procedure: streptavidin-biotin-peroxidase technique, hemalum counterstain, original magnification ×400. c) Distribution of repp86 LI (%) in OSCC as determined by immunohistochemistry.

Abbildungen 1a bis 1c. a) Normale Mukosa der Mundhöhle mit Repp-86-Färbung; Negativkontrolle rechts unten. Färbeprotokoll:Streptavidin-Biotin-Peroxidase-Technik, Hämalaun-Gegenfärbung; Originalvergrößerung 400×. b) Plattenepithelkarzinom mit Repp86-Färbung; Negativkontrolle rechts unten. Färbeprotokoll: Streptavidin-Biotin-Peroxidase-Technik, Hämalaun-Gegenfärbung; Originalvergrößerung 400×. c) Verteilung des Repp86-Labeling-Index (%) in Plattenepithelkarzinomen nach immunhistochemischer Färbung.

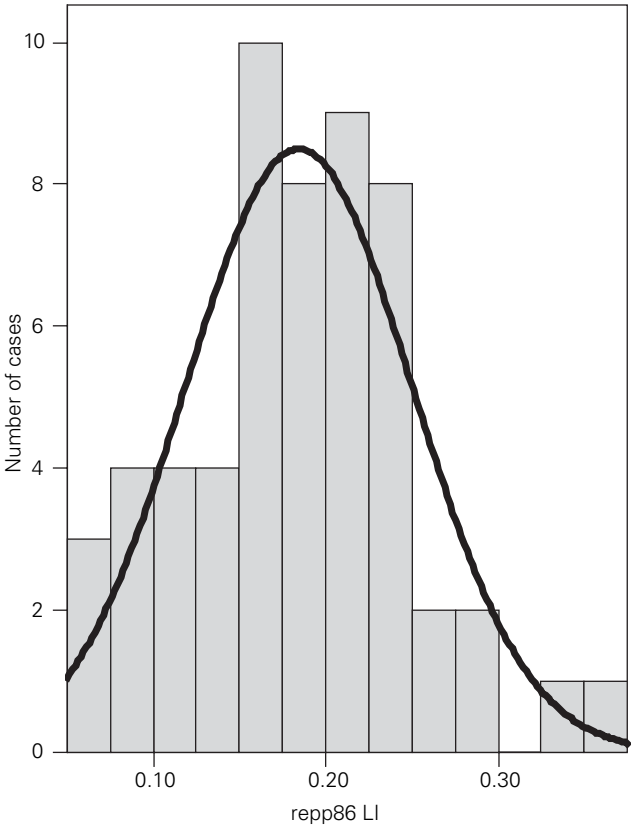

Figure 1c – Abbildung 1c

Hematopathology, University of Kiel, Germany) in a moist chamber at room temperature for 1 h [13, 22]. The secondary antibody consisted of a polyclonal, biotinylated rabbit-anti-mouse antibody (E0464, DAKO, dilution 1 : 50 in TBS, 30 min, room temperature). To provide subsequent chromogenic assay, incubation was carried out with avidin-biotin/horseraddish-peroxidase complex for 30 min (StreptABComplex/HRP, K0377, DAKO). The assay was obtained with AEC ("AEC+" Substrate-Chromogen System, K3469, DAKO). To enhance the contrast and improve evaluation, the nuclei were counterstained with hemalum (S3309, DAKO). The same procedure was applied for immunohistochemical staining of Ki-67 antigen and topoisomerase IIα. For Ki-67 immunostaining, we used the antibody Ki-S5, a monoclonal mouse IgG (dilution 1 : 100 in TBS, Department of Pathology and Hematopathology, University of Kiel, Germany). For topoisomerase IIα immunostaining, a commercially available monoclonal goat antibody (N-16, dilution 1 : 100 in TBS, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used.

From each tissue sample two sections were obtained consecutively and processed on microscopic slides, with one serving as a negative control in each case (identical treatment, but replacement of the incubation step with a primary antibody by incubation with TBS). Tissue sections of lymphatic tissue were included in each staining series as a positive control [17, 22].

Qualitative and Quantitative Analysis

Slides were examined qualitatively under a bright-field microscope (Axioskop, Zeiss, Oberkochen, Germany) with 100–400× magnification for changes in the extent and localization of repp86 expression. This was determined descriptively and compared with the controls. For quantification of repp86 expression, the LI was determined as ratio of positively expressing cells and total number of cells per visual field. For this, randomized systematic subsampling was used [2, 27]. Three visual fields per section for each sample were digitized with 400× magnification using a CCD camera (Axiocam, Zeiss) and the program Axiovision 3.1 (Zeiss). Counting was carried out per cm^2 using a grid template, evaluating 300 ± 50 cells median per visual field so that the overall number of evaluated cells with three visual fields per section was 900 ± 150 cells [28, 32]. The evaluation was carried out independently by two examiners. The criterion for positively stained cells was the existence of a clear cell structure with a clear specific staining of the nucleus. Staining of the extracellular matrix was not evaluated [13, 22].

Statistical Analysis

Descriptive analysis of LI data was performed using the arithmetic mean and standard deviation (SD). The Kolmogoroff-Smirnoff test was used to test for normal distribution. Correlations between continuous variables were analyzed by means of the Spearman rank-correlation coefficient. The Kruskal-Wallis nonparametric analysis of variance was used to characterize relationships between categorical and continuous variables. Two-sided adjusted p-values of $p \leq 0.05$ were considered to be significant. All calculations were made using SPSS 12.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Immunohistochemical Analyses

A total of ten samples of normal oral mucosa and 59 samples of OSCC of all stages were analyzed in a blind-trial fashion by immunostaining repp86 using the monoclonal antibody Ki-S2. Immunohistochemical staining of normal oral mucosa is shown in Figure 1a. About 4% of cells show positive nuclear staining. This fraction is located in the basal and suprabasal cell layers of the epithelium. In normal oral mucosa, repp86 LI ranged from 3% to 7%, the average expression was 4.7% (SD 1.7%). Immunohistochemical staining of a moderately differentiated squamous cell carcinoma of the floor of the mouth is shown in Figure 1b. About 20% of tumor cells show positive nuclear staining. Mitotic figures stain strongly. No staining of the cytoplasm or surrounding fibrous tissue is noted. In the whole series, immunolabeling for repp86 was consistently nuclear. Positive cells were often arranged in small clusters, but a diffuse distribution was also found. In OSCC, repp86 LI ranged from 5% to 37%. The Kolmogoroff-Smirnoff test showed normal distribution of repp86 LI ($p = 0.903$). The mean LI was 18.4% (SD 6.6%). Figure 1c shows the distribution of repp86 LI in OSCC.

Ki-67 and topoisomerase IIα LIs were highly variable as well. The average Ki-67 LI was 6.6% in normal mucosa (range:

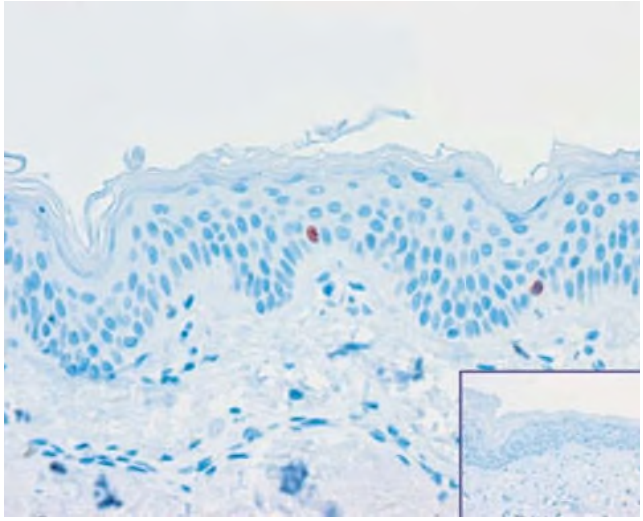

Figure 2a – Abbildung 2a

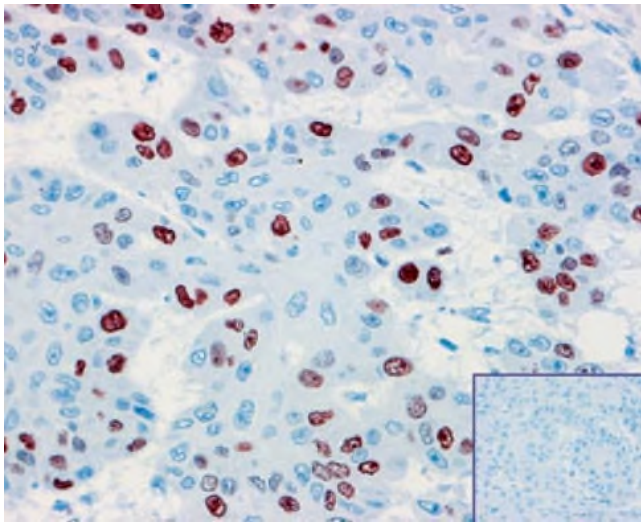

Figure 2b – Abbildung 2b

Figures 2a and 2b. a) Normal mucosa of the oral cavity with staining for Ki-67 and negative control. Staining procedure: streptavidin-biotin-peroxidase technique, hemalum counterstain, original magnification ×400. b) OSCC with staining for Ki-67 and negative control. Staining procedure: streptavidin-biotin-peroxidase technique, hemalum counterstain, original magnification ×400.

Abbildungen 2a und 2b. a) Normale Mukosa der Mundhöhle mit Ki-67-Färbung; Negativkontrolle rechts unten. Färbeprotokoll: Streptavidin-Biotin-Peroxidase-Technik, Hämalaun-Gegenfärbung; Originalvergrößerung 400×. b) Plattenepithelkarzinom gefärbt mit Ki-67-Färbung; Negativkontrolle rechts unten. Färbeprotokoll: Streptavidin-Biotin-Peroxidase-Technik, Hämalaun-Gegenfärbung; Originalvergrößerung 400×.

4–12%; SD 2.6%) and 26.7% in OSCC (range: 7–53%; SD 10.0%) Immunohistochemical staining for Ki-67 in normal oral mucosa and OSCC is shown in Figures 2a and 2b. For topoisomerase IIα we found a mean LI of 12.3% in normal mucosa (range: 4–20%; SD 5.5%) and 40.4% in OSCC (range: 13–71%; SD 13.7%). Topoisomerase IIα immunostaining is demonstrated by Figures 3a and 3b.

Correlation of Labeling Indices with Clinical and Histopathologic Parameters

Repp86 LI in relation to different groups defined by clinical and histopathologic characteristics is surveyed in Table 1. We found no correlation between repp86 LI and tumor stage/lymph node invasion or histopathologic grading. Ki-67 did not show a correlation with tumor stage/lymph node invasion or histopathologic grading either. While topoisomerase IIa expression was significantly correlated with tumor size ($p < 0.05$), a correlation with lymph node invasion, or histopathologic grading could not be observed.

To determine whether repp86 expression was related to Ki-67 and topoisomerase IIα levels, a correlation analysis was performed. Repp86 expression was positively correlated with Ki-67 index ($r = 0.48$; $p < 0.01$) as well as with topoisomerase IIα ($r = 0.39$; $p < 0.01$). Furthermore, we found repp86 expression to be negatively correlated with patient age at diagnosis, although this was only borderline significant ($r = –0.25$; $p = 0.06$). Ki-67 and topoisomerase IIα levels were also significantly correlated with each other ($r = 0.34$; $p < 0.05$) but not with age ($r = –0.068$ and $r = –0.049$, respectively). The results of the correlation analysis are shown in Table 2.

Notwithstanding the statistical significance found for some of these correlations, a broad range of LIs were encountered in all of the above-defined categories and the ranges were not strikingly different. These data indicate that the different grades and stages of OSCC are heterogeneous with respect to cellular proliferation [15].

Discussion

Repp86 could be detected immunohistochemically in all samples of oral mucosa and OSCC. The staining was strictly nuclear and mitotic figures stained strongly. Repp86 LI was significantly elevated in samples of OSCC compared to normal oral mucosa. These findings are in accordance with other studies on repp86 function and expression in different tumor entities [1, 17, 22, 23, 25]. Rudolph et al. reported a median repp86 LI of 9% (range: 0–29%) in a cohort of 371 women with lymph node-negative breast cancer [22]. Krams et al. found a mean repp86 LI of 10.2% (range: 0–48%; SD 9.9%) in a series of 161 children with neuroblastoma [17]. In squamous cell carcinoma, repp86 expression has not been reported yet (review of PUBMED database, November 20, 2004). In our investigation, we found a mean repp86 LI of 18.4% which is about twice as high as reported for neuroblastoma and breast cancer. However, considering the range of labeling values, our results are comparable to those reported by the trials mentioned above. Based on these results, it appears as though the frac-

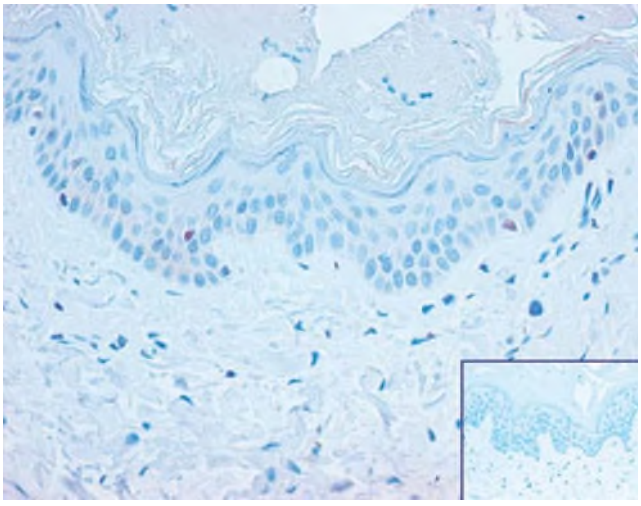

Figure 3a – Abbildung 3a

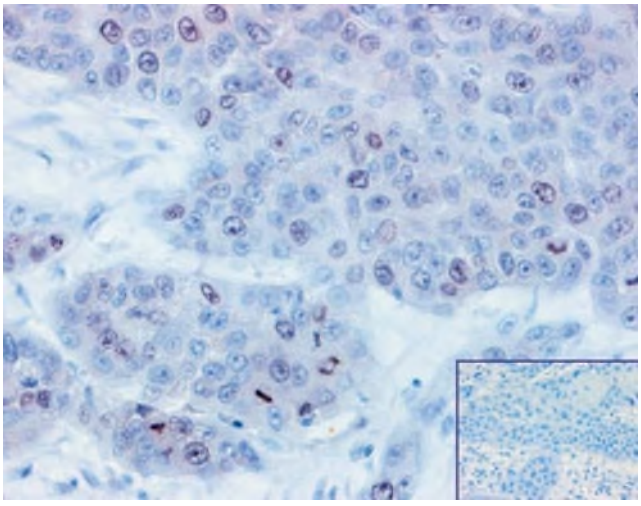

Figure 3b – Abbildung 3b

Figures 3a and 3b. a) Normal mucosa of the oral cavity with staining for topoisomerase IIα and negative control. Staining procedure: streptavidin-biotin-peroxidase technique, hemalum counterstain, original magnification ×400. b) OSCC with staining for topoisomerase IIα and negative control. Staining procedure: streptavidin-biotin-peroxidase technique, hemalum counterstain, original magnification ×400.

Abbildungen 3a und 3b. a) Normale Mukosa der Mundhöhle mit Topoisomerase-IIα-Färbung; Negativkontrolle rechts unten. Färbeprotokoll: Streptavidin-Biotin-Peroxidase-Technik, Hämalaun-Gegenfärbung; Originalvergrößerung 400×. b) Plattenepithelkarzinom mit Topoisomerase-IIα-Färbung; Negativkontrolle rechts unten. Färbeprotokoll: Streptavidin-Biotin-Peroxidase-Technik, Hämalaun-Gegenfärbung; Originalvergrößerung 400×.

Table 1. Repp86 indices in relation to tumor size, lymph node invasion, and histopathologic grading. SD: standard deviation.

Tabelle 1. Repp86-Labeling-Index in Relation zu Tumorgröße, Lymphknotenbeteiligung und histopathologischem Grading. SD: Standardabweichung.

		Repp86 labeling index				
		n	Mean	Minimum	Maximum	SD
Normal mucosa		10	4.7%	3%	7%	1.7%
Oral squamous cell carcinoma		59	18.4%	5%	37%	6.6%
Tumor size	T1	2	26%	17%	37%	14.3%
	T2	21	17.5%	9%	33%	6.2%
	T3	2	21%	21%	22%	0.7%
	T4	23	18.0%	7%	30%	6.3%
Lymph node	N0	16	18.2%	7%	37%	8.2%
	N1	12	18.4%	9%	26%	6.2%
	N2	20	18.4%	9%	30%	5.6%
	N3	0	–	–	–	–
Grading	G1	4	18%	7%	30%	9.7%
	G2	35	18.0%	7%	33%	5.8%
	G3	9	20%	9%	37%	8.5%

tion of cycling cells, as defined by repp86 expression, accounts for a larger proportion of the cell population compared to other tumor entities. As our results are derived from a limited number of cases, a further analysis of a larger cohort of patients is necessary.

Recent progress in analyzing the function and structure of cell cycle-associated proteins has considerably promoted our understanding of the mechanisms controlling cellular proliferation [19, 26]. Yet, among the large number of proteins involved, only few have proven useful for monitoring proliferative activity and tumor growth. The ability to identify antigens that are reliably associated with cellular proliferation in normal and neoplastic tissue, such as Ki-67 antigen and topoisomerase IIα, has proven valuable in diagnostic histopathology and cell biology [20, 29, 33]. However, in OSCC, the application of proliferation markers as indicators of prognosis has prompted conflicting results. Consequently, the assessment of proliferative activity has not been incorporated into broad clinical routine and decision processes in treatment of OSCC [4, 16, 18, 30]. A major shortcoming in assessing proliferative activity by Ki-67 and topoisomerase IIα immunostaining may be the inevitable inclusion of G_1-phase cells. The G_1-phase can be easily influenced by several external and internal factors. Cells in G_1-quiescence may pass to the restriction point G1/S and contribute directly to the magnitude of the proliferation compartment. Alternatively, they may senesce in G_0 or succumb by apoptosis. As cells in G_1 make up the largest fraction of the cycling subpopulation, G_1 is a major source of uncertainty in assessment of tumor proliferation [19].

Table 2. Correlation analysis of repp86, Ki-67, topoisomerase (Topo) IIα expression, and age at diagnosis.

Tabelle 2. Korrelationsanalyse der Expression von Repp86, Ki-67, Topoisomerase (Topo) IIα und dem Alter bei Diagnosestellung.

		Repp86	Ki-67	Topo IIα	Age
Repp86	Correlation (r)	1	0.476	0.394	–0.253
	Significance	–	0.001	0.003	0.060
	n	59	59	59	59
Ki-67	Correlation (r)	0.476	1	0.341	–0.068
	Significance	0.001	–	0.012	0.609
	n	59	59	59	59
Topo IIα	Correlation (r)	0.394	0.341	1	–0.049
	Significance	0.003	0.012	–	0.727
	n	59	59	59	59
Age	Correlation (r)	–0.253	–0.068	–0.049	1
	Significance	0.060	0.609	0.727	–
	n	59	59	59	59

In our investigation, the anti-repp86 antibody labeled only two thirds (66.7%) of the Ki-67- and less than one half (44.1%) of the topoisomerase IIα-expressing cell population. Thus, the G_1-phase cells account for up to 50% of the growth fraction in OSCC. Although the size of the growth fraction is estimated correctly by Ki-67 and topoisomerase IIα immunostaining, the repp86 LI may reflect the biologically relevant fraction of proliferating cells more accurately.

To be of relevance, any proliferation marker has to show a correlation with clinical outcome. The critical question is whether an exclusion of G_1-phase cells yields any advantages in terms of assessment of prognosis compared to an evaluation of the entire growth fraction. In the cohort of 371 women with lymph node-negative breast cancer, Rudolph et al. reported repp86 LI to be the most statistically significant predictor of overall survival, disease-specific survival, and disease-free survival in multivariate analysis (all two-sided $p < 0.0001$) [22]. In the risk group defined by a repp86 LI of > 10%, mortality was increased up to 20-fold. In the retrospective investigation of 161 children with different stages of neuroblastoma, Krams et al. found repp86 LI of > 10% to significantly predict a shortened disease-free interval and an increased tumor mortality (both $p < 0.0001$) [17]. Moreover, in multivariate analysis, repp86 LI emerged as the most important predictor of event-free and disease-specific survival (both $p < 0.0001$). Both authors concluded that prognosis is apparently best indicated by the percentage of cells in S- through M-phases of the cell cycle, as determined by repp86 immunostaining. Although the prognostic value of repp86 expression has not been determined in OSCC yet, Fietkau et al. reported the S-phase fraction as determined by one-dimensional flow cytometry to be prognostically significant [5]. Due to the limited number of patients and the different arms of therapy (preoperative radiochemotherapy followed by tumor resection vs. tumor resection followed by radiotherapy or radiochemotherapy), we did not perform an analysis of outcome in this study.

Based on these results, a further investigation of the prognostic significance of repp86 expression in OSCC seems promising.

Conclusion

The results of our study show that the expression of repp86 is significantly elevated in OSCC, when compared to normal oral mucosa. Repp86 LI correlates with Ki-67 and topoisomerase IIα but not with tumor size, lymph node invasion, and histopathologic grading. Due to the nature of this preliminary study, clinical data on recurrence and survival were not incorporated in the analysis. On the basis of our findings, a further evaluation of repp86 expression in a larger series of cases with respect to prognosis is necessary.

Acknowledgments

We would like to thank Prof. Dr. Dr. Parwaresch, Department of Pathology and Hematopathology, University of Kiel, Germany, for the generous provision of Ki-S2 and Ki-S5 antibodies.
This study was supported by a grant of Johannes and Frieda Marohn Foundation (Grant No.: Fen/03).

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