

# Molecularbiology of Basal Cell Carcinoma

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## 1. Introduction

Basal cell carcinoma is the most frequently occurring skin tumor. Most cases are not life threatening. Only a very small proportion of BCCs metastasize. A high tendency to recurrence makes characterizing BCCs and tumor margin areas obligatory. It will assist in better understanding their pathogenesis and in more effective treatment through prevention of recurrence and second primary disease. In addition to histopathological assessment, the spread of the primary tumor according to TNM classification is crucial for estimating the further development of the disease.

### 1.1 Molecular alterations involved in the emergence and development of basal cell carcinomas (BCC)

While it has not been fully clarified which primary cell gives rise to basal cell carcinomas it is supposed that BCCs arise from interfollicular basal cells, keratinous cells of hair follicles or sebaceous glands (1 to 6). Chemically induced BCCs were produced in the pilosebaceous structures of rats (3, 7). Solar ultraviolet radiation (UVR), in particular ultraviolet B (UVB, waveband 280-315 nm), is a major and significant factor (8 to 23) in many of the carcinogenic steps leading to basal cell carcinomas. Additional risk factors are predisposing syndromes and genetic predisposition (3, 11 to 14, 16, 19, 24 to 41), such as skin-type (13, 42, 43), viral infections, above all human papilloma viruses (HPV) and / or immunosuppression (3, 13, 17, 20, 35, 39, 41, 44 to 46). The influence of UVB is evident from the fact that BCC incidence is lowest in Finland (47, 48) and highest in Australia (13, 47 to 51). Incidence increases with advancing age through the accumulation of UV-radiation (52, 53). UVB exposure and chronic oxidative stress (54) effect direct DNA damage, mutations and chromosome aberrations in the skin (41, 55 to 57), all of which are found in basal cell carcinomas. They are comparable with squamous cell carcinomas (58) insofar as field cancerization can be shown in the environment of the BCC (59 to 63). The BCC can be characterized by certain markers such as proof of p53. Figure 1 illustrates UVB damage with regard to the mutation and inactivation of tumor suppressor gene p53 as well as activation of telomerase during cancerization, particularly in basal cell carcinomas (64).

The inactivation of tumor suppressor activity is a universal step in the development of human cancers (65, 66). With inactivation p53 loses its function as the “guardian of the genome” (66) thanks to which it normally controls, among other things, the normal cell

cycle, arresting irregularly growing cells via G1 / G2 transition of the cell cycle and compelling them to programmed suicide, apoptosis (67 to 76). bcl-2 can block and control p53-dependent apoptosis and loss of bcl-2 stimulates apoptosis (67 to 71, 73, 77, 78). While wild-type p53 suppresses cell growth, p53 mutations reduce DNA repair, for example after UVB radiation, and stop apoptosis. That can explain the increased incidence of skin tumors such as basal cell carcinoma through UVB radiation (13, 79 to 82).

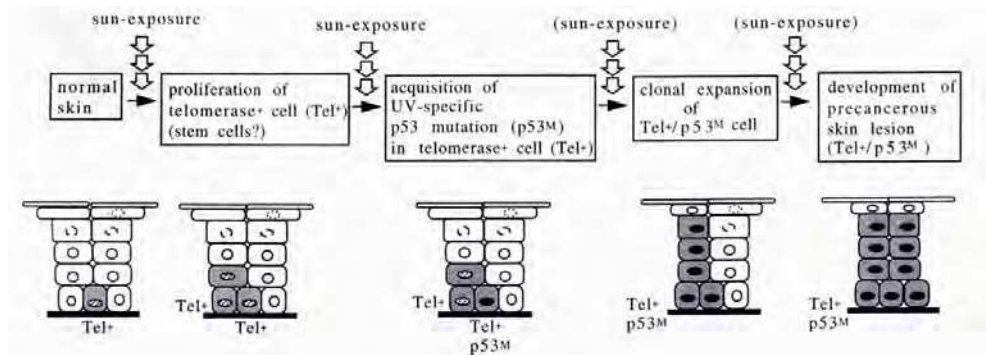


Fig. 1. Carcinogenesis of basal cell carcinomas. Concept for the course of molecular changes in the cancerogenesis of basal cell carcinomas: adapted and reprinted by permission of the American Association for Cancer Research: Ueda et al. (64) (Tel+ = telomerase activation).

## 1.2 Molecular markers for differentiation of BCC and SCC of the skin

Basal cell carcinomas and squamous cell carcinomas of the skin are differentiated by means of histopathological staining, mostly with hemalaun-eosin staining. This can lead to difficulty, as seen for instance in cytology. Vega-Memije et al. (83) introduced Papanicolaou staining to successfully distinguish between the two carcinoma types, and this was also used by Christensen et al. (84) along with May-Grünwald-Giemsa staining for the cytological differentiation of BCCs and actinic keratosis. Tellechea et al. (85) used immunohistochemical anti-Ber-EP4 monoclonal antibody. The total of 22 BCCs showed positive reactions, while in 21 SCCs the reaction was negative, proving this monoclonal antibody suitable for differentiating between the two nonmelanomatous skin-tumors. It was confirmed by Beer et al. (86) who verified expression of Ber-EP4 in all of 39 BCCs and in none of 23 SCCs tested. Swanson et al. (87) were able to distinguish BCCs both from SCCs of the skin and from trichoepitheliomas (TE) with proven expression of Ber-EP4 (also of bcl-2 and CD34): The reaction of Ber-EP4 was positive in all of 44 BCCs, in 81% (29/36) of TEs and in none of 22 SCCs. According to the same procedure, Tope et al. (88) differentiated between BCC, actinic keratosis (AK) and SCCs: All of 5 superficial BCCs were Ber-EP4 positive, while all of 10 AKs and of 8 SCCs were negative. To differentiate between BCCs and SCCs the Ber-EP4 marker is the most suited. Swanson et al. (87) differentiated with CD34 BCCs, SCCs and TEs: CD34 was positive in 16% (7/43) of BCCs, in 25% (5/20) of SCCs and in 8% (3/36) of TEs. Likewise, Yada et al. (89) and Aiad and Hanout (90) were able to discriminate BCC from SCC immunohistochemically with CD10. In the study by Yada et al. (89) BCCs expressed CD10 1+ to 2+ in 86% (44/51) and in all 9 SCCs CD10 was negative. According to

Aiad and Hanout (90) CD10 expression was significantly higher in BCCs than in SCCs: 10/21 (48%) and 0/16 SCC ( $p=0.002$ ).

Guttinger et al. (91), Seelentag et al. (92), Dietrich et al. (93) and Seiter et al. (94) used immunohistochemical methods with monoclonal anti-CD44s and various anti-CD44 splice variant antibodies to differentiate BCCs and SCCs. Guttinger et al. (91) obtained distinct marking of tumor cells (++) in all SCCs with anti-CD44s, CD44v4, CD44v6 and CD44v9 antibodies. The nodular BCCs showed no expression and the sklerodermiform BCCs (+) a weaker expression. This result was congruent with studies by Seelentag et al. (92). Seelentag et al. observed the expression of CD44s, CD44v3, CD44v4, CD44v6 and CD44v9 in immunohistochemical paraffin sections. They (92) achieved higher or distinctly higher expression of CD44v3, CD44v5, CD44v6 and CD44v9 in SCCs of the skin ( $n=37$ ) than in BCCs ( $n=10$ ). In BCCs the expression was low (CD44s, CD44v3) or absent (CD44v4). Higher expression in BCCs was found with the splice variants CD44v5, CD44v6 and CD44v9. In squamous cell carcinomas Seelentag et al. (92) found CD44v3, CD44v5, CD44v6 and CD44v9 expression comparable with that in keratoacanthomas ( $n=12$ ). CD44s and CD44v4 expression was lower in SCCs than in keratoacanthomas. This contradicted the findings by Dietrich et al., (93) who demonstrated CD44v4, CD44v5 and CD44v6 both in BCCs ( $n=7$ ) and in SCCs ( $n=6$ ). Indeed, using immunohistochemical methods Dietrich et al (93) demonstrated expression of CD44v7/8 and CD44v10 in all BCCs but in none of the SCCs tested. Forming groups according to the estimated percentage of staining cells, Seiter et al. (94) assessed expression of CD44s, CD44v5, CD44v6, CD44v7, CD44v7/8 and CD44v10 semiquantitatively. In the BCCs and SCCs they investigated, CD44s was expressed in the same degree, while all splice variants tested showed higher expression in SCCs. Simon et al. (95) checked the suitability of immunohistochemistry for definite distinction between BCCs and SCCs with splice variants CD44v3, CD44v4, CD44v5, CD44v6, CD44v7/8 or CD44v10. All splice variants stained the tumor center of BCCs, while in SCCs only tumor periphery was marked. 12/17 BCCs (71%) were positive with CD44v3 and 13/17 (77%) with CD44v5. The remaining CD44-splice variants marked all BCCs, and all of 16 SCCs were marked with all CD44-splice variants. In an immunohistochemical study by Son et al. (96), 49% of BCCs and only 35% of SCCs showed CD44v6 marking.

Al-Sader et al. (97) sought to differentiate immunohistochemically the proliferative indices between BCCs and SCCs. The median scores of the mitotic index were 5 for BCCs and 4 for SCCs ( $p=0.621$ ), but the authors (97) observed that the median scores of Ki-67 were significantly different, amounting to 271 (per 1000 cells) in BCCs and 340 (per 1000 cells) in SCCs ( $p=0.029$ ). To differentiate BCCs from skin-SCCs, Park et al. (98) used immunohistochemical proof of p53, p63 and survivin. With p53 the authors were unable to differentiate between the two, which were malignant in differing degrees: 90% 2+ to 3+ vs. 100% 2+ to 3+. Proof of p63 made this distinction possible: BCC 90% 2+ to 3+, SCC 40% 2+, as did proof of survivin (BCC 40% 2+, SCC 80% 2+ to 3+). However, Bäckvall et al. (99) succeeded in demonstrating a significant difference between normal skin adjacent to BCC and that adjacent to SCC ( $p<0.05$ ) with regard to the number of p53 clones. Chang et al. (100) found significantly less Ki-67 in 10 BCCs investigated (mean score value  $12\pm7$ ) than in 8 SCCs (mean score value  $47\pm21$ ), ( $p<0.05$ ), and detected clearly different expression of p53 (mean score value BCC  $50\pm17$  vs. SCC  $61\pm15$ ). Son et al. (96) used

immunohistochemical findings of Ki-67 and p53 to differentiate BCCs (n=108) from SCCs (n=94). Ki-67 expression was low in 90% of BCCs and high in 10%, while Ki-67 showed high expression in 27% of SCCs and low expression in 73%. p53 was demonstrated in 15% of BCCs and in 46% of SCCs. Bolshakov et al. (101) succeeded in establishing clear differences between aggressive SCCs / nonaggressive SCCs and also aggressive BCCs / nonaggressive BCCs through mutation frequency of p53 with single-strand conformation polymorphism and sequencing: frequency of p53 mutations amounted to 35% (28/80) of aggressive SCCs vs. 50% (28/56) of nonaggressive SCCs as opposed to 66% (35/50) of aggressive BCCs vs. 38% (37/98) of nonaggressive BCCs. Chang et al. (100) also demonstrated a clear differentiation between BCC and SCC by immunohistochemical bcl-2 detection: All of 10 BCCs displayed positive marking that was absent in all of 8 SCCs. In consonance with this, Delehedde et al. (80) found immunohistochemically distinct bcl-2 expression (+++) in 17 BCCs examined, which was missing in all of 14 SCCs ( $p < 0.05$ ). Likewise, Swanson et al. (87) found positive bcl-2 reaction in 91% (41/45) of BCCs and in 18% (4/22) of SCCs. Contrary to these results Al-Sader et al. (97) observed no difference between the median scores of the apoptotic index in BCCs (10.5 apoptotic cells / 1000 cells) and in SCCs (10 apoptotic cells / 1000 cells).

In our studies we introduced proof of telomerase activity to differentiate BCCs from SCCs (102 to 104), cf. 2.4.1: the proportion of BCCs with activation of telomerase amounted to 87% (26/30) and was barely higher than in SCCs of the skin with 75% (9/12). This result is in approximate conformity with synoptic references: activation of telomerase in 87% (177/203) of BCCs examined altogether and in 77% (58/75) of SCCs of the skin (103, 104). However, our studies demonstrated that the score values of immunohistochemical proof with APAAP (105) of the telomerase subunit hTERT cannot be used to differentiate between BCCs (n=24) and SCCs (n=7) of the skin (106, 107): BCC mean score value  $9.4 \pm 4.9$  (Ab 1: anti-hTERT antibody Calbiochem, USA, code 582005) and  $9.0 \pm 4.0$  (Ab 2: anti-hTERT antibody code NCL-hTERT Novocastra, UK, clone 44F12,) vs. SCC mean score value  $9.9 \pm 2.4$  (Ab 1) and  $9.1 \pm 3.8$  (Ab 2), cf. 2.4.2.

## 2. Molecular markers for basal cell carcinomas and tumor-free margin tissues

Adequate resection of BCCs is of utmost importance in preventing recurrence (108 to 114). Other authors are therefore searching for markers which discriminate between basal cell carcinoma and histopathologically tumor-free margin. In addition to histopathological assessment, assessing the spread of the primary tumor ( $T1 < 2\text{cm}$ ,  $T2 \geq 2\text{cm}$ ) according to the TNM classification (115) is crucial for assessing further development of the disease. The risk of recurrence of each tumor is defined as closely as possible to enable choice and application of the most appropriate clinical procedure (51, 116, 117). Tumor size is important for prognosis of the disease, as is shown by comparison in Kaplan-Meier curves. With all due precaution given the many censored cases, we ascertain that patients with primary T2 tumors relapse much earlier than those with T1 tumors (Log-rank  $p = 0.003$ ): after  $14 \pm 2$  months (T2) vs.  $65 \pm 16$  months (T1), Figure 2.

The classification in nonaggressive basal cell carcinomas and aggressive BCCs is predominantly determined by the clinical course of BCCs and by the tendency to recurrence: nonaggressive nodular, micronodular or superficial BCCs and aggressive infiltrative or

morphea BCCs (20, 81, 118 to 121). Additional markers will help to characterize and are essential for distinctly determining the spread of BCCs (19).

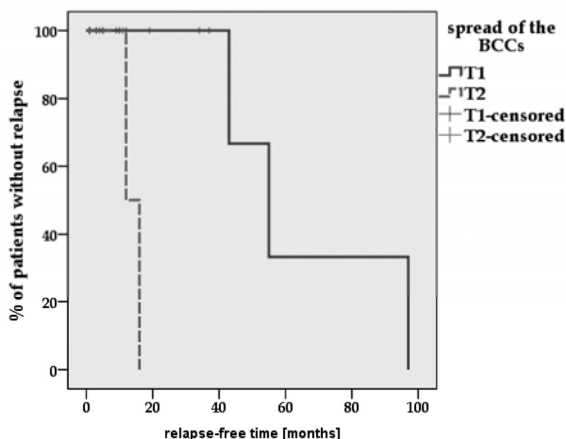


Fig. 2. Up-to-date outcome in patients with BCCs and the spread of the tumor (T1 < 2cm, T2  $\geq$  2cm) in Kaplan-Meier curves.

## 2.1 Different markers classifying nonaggressive and aggressive BCCs

Staibano and colleagues classified between nonaggressive BCC1 without relapse and aggressive BCC2 with local recurrence during metaphylaxis. For this differentiation they introduced various markers. They detected high expression of p53 in 60% of 30 BCC2 tumors and low expression in 10% of BCC1 tumors (122), or in 0/21 BCC1 tumors and in 82% (18/22) of BCC2 tumors, respectively (123). Bolshakov et al. (101) were also able to discriminate clearly between aggressive BCCs and nonaggressive BCCs through determination of p53 mutations by single-strand conformation polymorphism and nucleotide sequencing: the frequency of p53 mutations was 66% (33/55) with aggressive BCCs and only 38% (37/98) with nonaggressive BCCs. In 11 aggressive BCCs Ansarin et al. (121) found significant immunohistochemical nuclear staining of p53 in more than 50% of tumor cells and in 33 nonaggressive BCCs in less than 50% of tumor cells ( $p < 0.01$ ).

In BCC1 tumors the AgNOR scores were  $6.56 \pm 1.98$  and in BCC2-tumors  $9.48 \pm 2.12$ : De Rosa et al. (119). When Staibano et al. (124) ascertained the apoptotic index, they found a marked difference: BCC1  $5.98 \pm 2.52\%$  vs. BCC2  $39.82 \pm 8.32\%$ . BCC1 tumors examined showed a distinct cytoplasmic staining of bcl-2. This marker was missing in all of the 30 BCC2 tumors examined by Staibano et al. (122). In agreement with these investigations Ramdial et al. (81) detected a distinctly higher bcl-2 expression in immunohistochemical assays in nonaggressive BCCs than in aggressive BCCs: 45/50 (90%) nonaggressive BCCs expressed bcl-2 2+ to 4+ and 22/25 (88%) aggressive BCCs maximally 2+. High expression of cyclin D1 was not found in any BCC1 tumors but in all of 30 BCC2 tumors (125). By determining DNA ploidy Staibano et al. (125) were also able to differentiate between BCC1 and BCC2: All BCC2 tumors were aneuploid as against 77% of BCC1 tumors. After

immunohistochemical staining for monoclonal antibody against factor VIII (von Willebrand factor), microscopic counts of microvessels revealed a statistically significant difference: mean score value BCC1  $25.76 \pm 2.21$  vs. BCC2  $46.76 \pm 2.54$  (126). Histopathological diagnostics using these markers can be supplementary evidence for the surgeon deciding on the surgical procedure for therapy.

## **2.2 Examination of BCC spread by means of anti-CD44 antibodies and anti-splice variant antibodies, by anti-E48 and anti-U36 antibodies**

Cell adhesion molecules such as CD44 splice variants can be used as selective markers for different tumors and are important both for the diagnosis and prognosis of some tumors (127 to 129). Squamous cell carcinomas with poor differentiation display downregulation of CD44v6 expression in comparison with squamous cell carcinomas with high differentiation. Hyckel et al. (130) planimetered the immunohistochemical reaction of oral SCCs by anti-CD44 monoclonal antibodies and confirmed an inverse relation to carcinoma grading. Our immunohistochemical findings in frozen sections with APAAP (105) in regard to squamous cell carcinomas of the head-and-neck region (131 to 133) are comparable with the downregulation of isoforms containing CD44v6 that Salmi et al. (134) observed with CD44v3 and CD44v6 in SCCs in the head-and-neck area.

Guttinger et al. (91), Baum et al. (135), Seelentag et al. (92), Seiter et al. (94), Simon et al. (95), Kooy et al. (136), Dingemans et al. (137, 138) and Son et al. (96) used immunohistochemical marking on BCCs with CD44v6 and found it better than hemalaun-eosin staining for identifying their local spread. In 20 BCCs, Seelentag et al. (92) found low or no expression of CD44s or CD44v4, contrary to SCCs with low expression of CD44v3. The authors found high expression of CD44v5, CD44v6 and CD44v9 in BCCs, but the expression was lower than in SCCs.

We had previously achieved better results discriminating squamous cell carcinomas of the head and neck from their surroundings with immunohistochemical APAAP (105) in small frozen sections by using adhesion molecules CD44v6 (also its chimera U36) and E48 (131 to 133). This method was now confirmed in BCCs. We accomplished APAAP (105, cf. 131, 132) with a monoclonal antibody against the adhesion molecule CD44v6 (clone VRR-18, Bender, Austria) and with two monoclonal antibodies against adhesion molecules E48 (Centocor B.V., Netherlands) and U36 (Centocor B.V., Netherlands). Antibody E48 has been developed to recognize normal squamous epithelium and squamous cell carcinomas (139). It is well suited for differentiating carcinomas from negative adenocarcinomas and small cell carcinomas (140). Monoclonal anti-U36 antibody is an anti-CD44v6-chimeric (mouse/human) antibody for marking normal human squamous epithelia and squamous cell carcinomas (141 to 143). The quantitative presentation of antigen expression (CD44v6, E48, U36) in frozen sections was evaluated in our study by means of an immunoreactive score (IRS) according to Remmele et al. (144) and Remmele and Stegner (145). Our evaluation was performed three times by an independent examiner. The membrane staining intensities (SI) were divided into 5 levels: negative, 1+ (weak expression) to 5+ (very strong expression). The percentage of positive stained cells (PP) was classified into 4 groups according to percentage of stained cells: 0-10%=score 1; 10-50%=score 2; 50-75%=score 3; 75-100%=score 4. The IRS was calculated by multiplication of SI and PP. To identifying the spread of BCCs in small frozen sections we applied immunohistochemical

staining with APAAP (105) to membrane marking of CD44v6, E48 and U36. We found it better than hemalaun-eosin staining.

In our study the immunoreactive scores (IRS) in BCC tumor center vs. tumor-free margin were demonstrated and are illustrated in Figure 3. Only the expression of CD44v6 was significantly higher in tumor center (mean score value  $12.5 \pm 3.2$ ) than in squamous epithelia in the tumor margin (mean score value  $8.4 \pm 4.0$ ,  $p=0.006$ ), and was comparable to the expression of Ki-67 ( $p<0.001$ ). Microscopic representation with the three monoclonal antibodies, particularly CD44v6, distinguished BCCs clearly from their surroundings: Figure 4.

The ample expression of CD44v6 and U36 (if necessary E48) adhesion molecules might make them useful both for the diagnostics and adjuvant therapy of BCCs. Radionuclide-labeled purified monoclonal antibodies were successfully developed in VU University Medical Center (Amsterdam/Netherlands). They have proven themselves in this capacity for years in the diagnostics (146 to 152) and therapy (142, 147, 153 to 156) of squamous cell carcinomas in the head-neck-region.

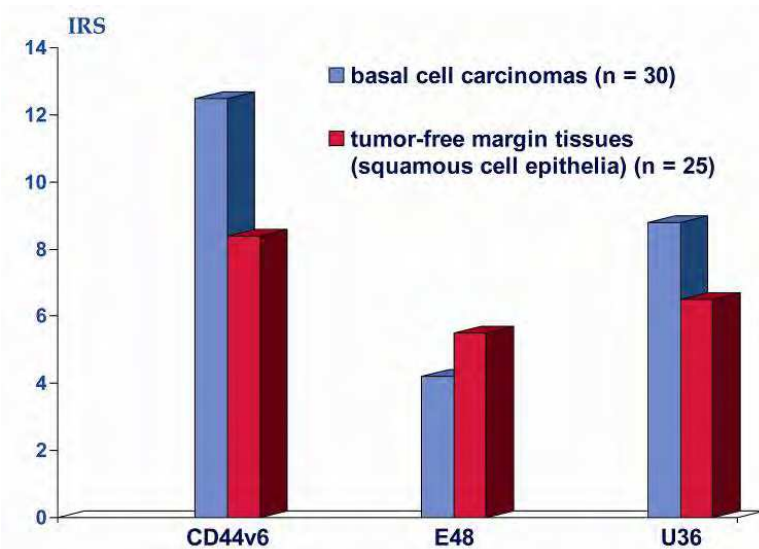
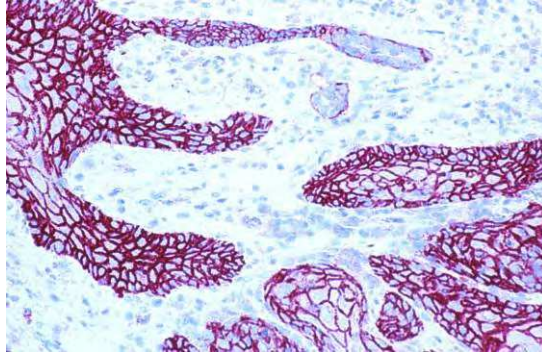
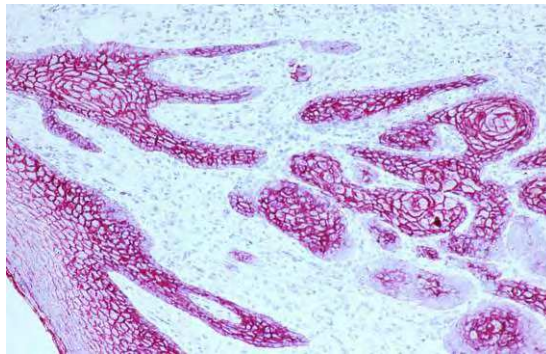


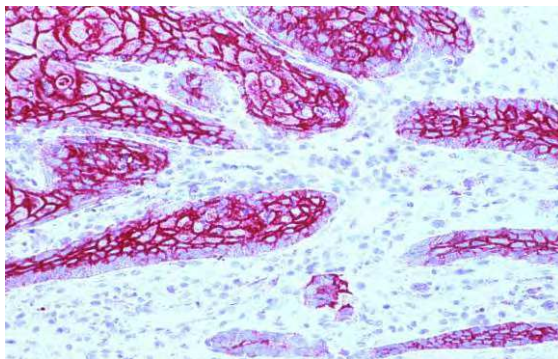
Fig. 3. Immunoreactive scores (IRS, cf. 144, 145) of expression of CD44v6, E48 and U36 in basal cell carcinomas (mean values).



a) CD44v6



b) E48



c) U36

Fig. 4. Comparison of expression of CD44v6 (a), E48 (b) and U36 (c) in small frozen sections of BCCs, magnification 200x.



### 2.3 Markers p53 and Ki-67 in BCCs and BCC-free tumor margin tissue

Molecular alterations can be found during carcinogenesis and tumor growth. As illustrated schematically in Figure 1 (cf. 1.1), the inactivation of tumor suppressor gene p53 is an important step towards carcinogenesis. During inactivation and mutation of p53 mutated p53 protein is formed. Mutated and wildtype p53 protein can be detected immunohistochemically by different or identical antibodies, for instance in tissues with field cancerization after intensive solar irradiation (60, 62) as well as in tumor-free margin surrounding of BCCs (98, 157 to 161). Authors are therefore searching for markers such as p53 which discriminate between basal cell carcinoma and histologically tumor-free margin surrounding the tumor.

#### 2.3.1 Marker p53 in BCCs and BCC-free tumor margin tissues

Urano et al. (157) applied immunohistochemistry using anti-p53 antibody clone Do-7, which recognizes both wildtype p53 protein and mutated p53 protein. The authors found significantly lower p53 expression in tumor margin than in tumor center ( $p < 0.05$ ): 7/17 tissues of tumor margin (41%) and 11/17 (65%) of tumor center were p53 positive. Barrett et al. (158) were able to detect p53 in 20/27 (74%) BCCs. They also investigated adjacent actinic keratosis in the tumor margin of 4 tumors and found increased p53 staining. Demirkan et al. (159) detected significantly less p53 in BCC-free tumor margin than in the tumor center: 26% (11/42) p53 positive tumor-free margins vs. 44% (8/18) tumor center tissues ( $p = 0.034$ ), the same was found with monoclonal antibody Do-7 (DAKO/Denmark). According to investigations by Rajabi et al. (160), p53 expression in 96/123 BCC tumor tissues (82%) was not significantly higher than in 84/117 tumor-free margin tissues (78%),  $p = 0.38$ . The relatively high p53 expression of the tumor margin could be related to strong sun exposure of uncovered skin with patients in southern latitudes. Bäckvall et al. (99) and Koseoglu et al. (161) were also unable to find a significantly decreased expression of p53 in adjacent tissues surrounding the BCCs. Koseoglu et al. (161) detected p53 clones in 10/43 tumor center tissues (23%) and in 9/21 tumor margin tissues (42%).

In our study we employed an antibody from Oncogene (USA) to ascertain p53 scores in small frozen sections: a monoclonal antibody against mutated p53 Ab-3 (OP 29-1) (103, 106, 107). Before performing the APAAP (105), slides with frozen sections were fixed in methanol and acetone and pretreated in a steamer (95 to 99°C). This was non-essential for p53 and Ki-67 but for hTERT and retinoic acid receptors nuclear staining in frozen sections essential (106, 107, 162). We determined p53 scores as described in 2.2. In quantitative presentation of antigen expression (p53, Ki-67, hTERT and retinoid receptors) by means of an immunoreactive score (IRS) we refer exclusively to the nuclear and / or nucleolar staining. We evaluated staining intensity (SI) and number of positive stained cells (PP) in the BCCs and in squamous cell epithelia of the tumor margin. The IRS was calculated by multiplication of SI and PP. In our immunohistochemical investigations the difference between IRS of p53 in tumor center (mean score value  $7.9 \pm 3.4$ ) was not significantly higher than in tumor margin (mean score value  $5.8 \pm 4.2$ ),  $p = 0.095$ : Figure 5 and illustrated in Figure 6a (tumor center) and Figure 6c (tumor margin), cf. 2.3.2. p53 scores in T2-BCCs (8 primary tumors  $\geq 2$ cm) were not higher than in T1-BCCs (17 primary tumors  $< 2$ cm): mean score value  $6.3 \pm 4.3$  (T2) vs.  $7.9 \pm 3.3$  (T1),  $p = 0.328$ . We tested the role of p53 scores investigated for prognosis in Kaplan-Meier curves (cf. 2.3.2). We divided all p53 scores (also Ki-67 scores and

hTERT scores) into two groups (group 1: scores < mean value, group 2: scores  $\geq$  mean value) and used the patients' up-to-date outcome as documented in our hospital. With all due reservation due to the many censored cases, we ascertain that BCCs of patients with higher p53 scores in tumor center tissues recurred later, almost significantly later, than BCCs of patients with lower scores in tumor center tissues ( $65 \pm 16$  months vs.  $23 \pm 6$  months): Log-rank  $p=0.059$ , Figure 7a. However, patients with higher p53 scores in BCC tumor margin tissues did not relapse significantly but nevertheless earlier than patients with lower p53 scores in tumor margin tissues ( $36 \pm 6$  months vs.  $40 \pm 11$  months): Log-rank  $p=0.622$ , Figure 7c.

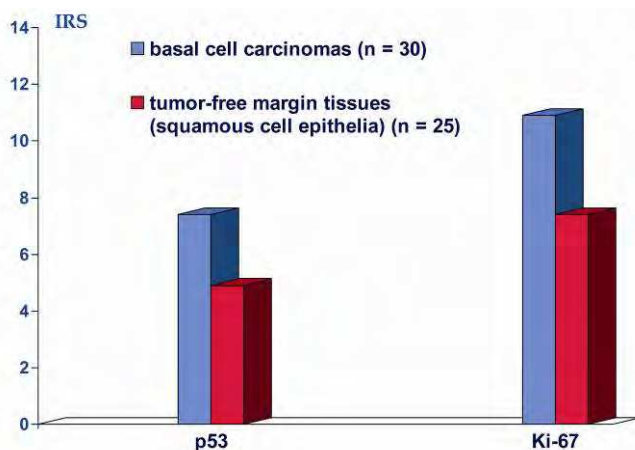


Fig. 5. IRS (immunoreactive scores, cf. 144, 145) mean values of expression of mutated p53 and of Ki-67 in basal cell carcinomas.

### 2.3.2 Marker Ki-67 in BCCs and BCC-free tumor margin tissues in comparison with p53

Some authors compared proof of p53 with expression of the proliferation marker Ki-67. Healy et al. (163) proved 71 BCC tissues immunohistochemically with p53 and Ki-67, 17 BCCs group 1 (patients without recurrence), 17 BCCs group 2-0 (patients who relapsed some time later) and 17 BCC relapses (group 2-R). p53 was demonstrated in 95% of each group. However, the authors (163) revealed significantly lower values of Ki-67 expression in BCCs in group 1 (mean value 12%) than in group 2-0 (mean value 25%) or group 2-R (mean value 22.5%):  $p=0.009$ . Barrett et al. (158) examined expression of p53, Ki-67 and PCNA in 27 basal cell carcinomas and compared them to histopathological BCC types. Expression of p53 and PCNA was higher in aggressive BCCs than in nonaggressive BCCs, and PCNA was higher than Ki-67. Chang et al. (100) tested 10 BCCs and detected both p53 and Ki-67 in all of them. The labeling index with Ki-67 was significantly lower than with p53:  $12 \pm 7$  vs.  $50 \pm 17$  ( $p < 0.05$ ). By contrast, in 20 BCCs Abdelsayed et al. (164) demonstrated higher marking with Ki-67 (mean value  $51.25 \pm 6.06$ ) and PCNA (mean value  $52.25 \pm 7.57$ ) than with p53 (mean value  $31.75 \pm 9.02$ ). Koseoglu et al. (161) also investigated the p53 level of 50 BCCs immunohistochemically in comparison with Ki-67 expression (anti-Ki-67 antibody clone Ki-88). They found significantly higher Ki-67 expression in p53 positive tissues (Ki-67 mean value 14.66) than in p53 negative tissues (Ki-67 mean value 8.37):  $p=0.019$ .

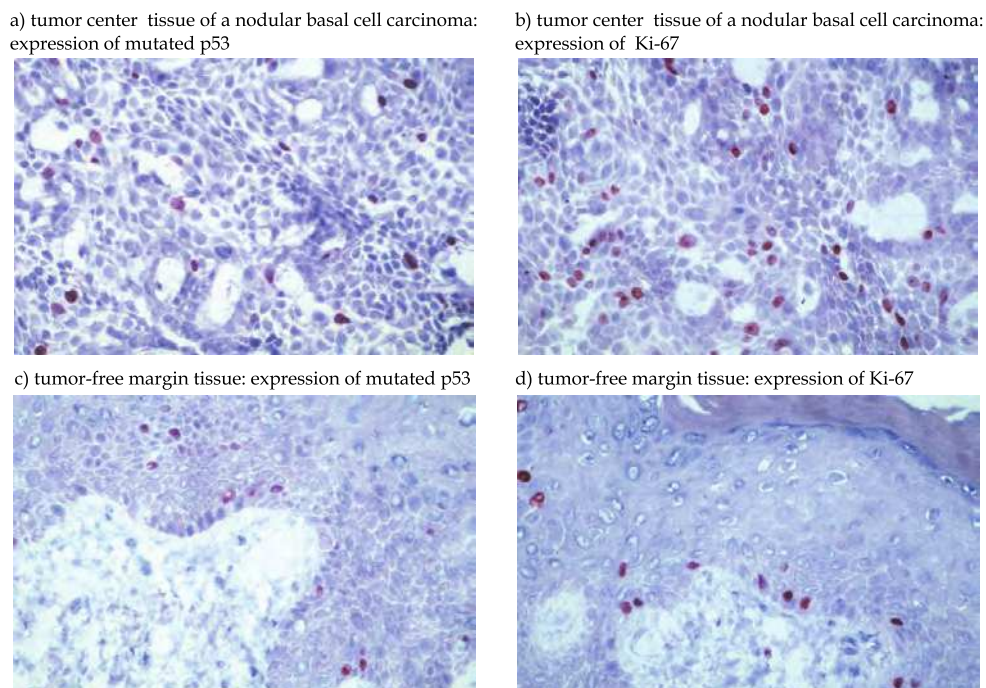


Fig. 6. Comparison of expression of p53 (a, c) and Ki-67 (b, d) in small frozen sections of BCCs (a, b) and in tumor margin tissues (c, d), magnification 400x.

We also compared Ki-67 scores with p53 scores in tumor center tissues and tumor-free margin tissues. To prove Ki-67 in small frozen sections we used a monoclonal mouse anti-human Ki-67 antibody (clone MIB-1) from DAKO (Denmark) (106, 107). As described under 2.2 and 2.3.1, nuclear staining scores were achieved with immunohistochemical APAAP (105). The Ki-67 mean score value in tumor center ( $10.9 \pm 2.5$ ) was significantly higher than that in tumor margin ( $7.4 \pm 1.8$ ),  $p < 0.05$ : Figure 5, cf. 2.3.1. Our results with p53 and Ki-67 expression of tumor center and tumor margin tissues are displayed in Figure 6.

We tested the prognostic prediction of Ki-67 status (107) detected in our patients' tissues with BCCs in Kaplan-Meier curves. With all due reservation in view of the number of censored cases, patients with higher Ki-67 scores in tumor center tissues suffered BCC recurrence (not significantly) later (Log-rank  $p = 0.560$ ) than patients with lower Ki-67 scores ( $56 \pm 16$  months, higher scores, vs.  $27 \pm 7$  months, lower scores): Figure 7b. In contrast, patients with higher Ki-67-scores in tumor margin tissues did not relapse significantly earlier (Log-rank  $p = 0.321$ ) than patients with lower Ki-67-scores ( $35 \pm 5$  months, higher scores, vs.  $48 \pm 13$  months, lower scores): Figure 7d.

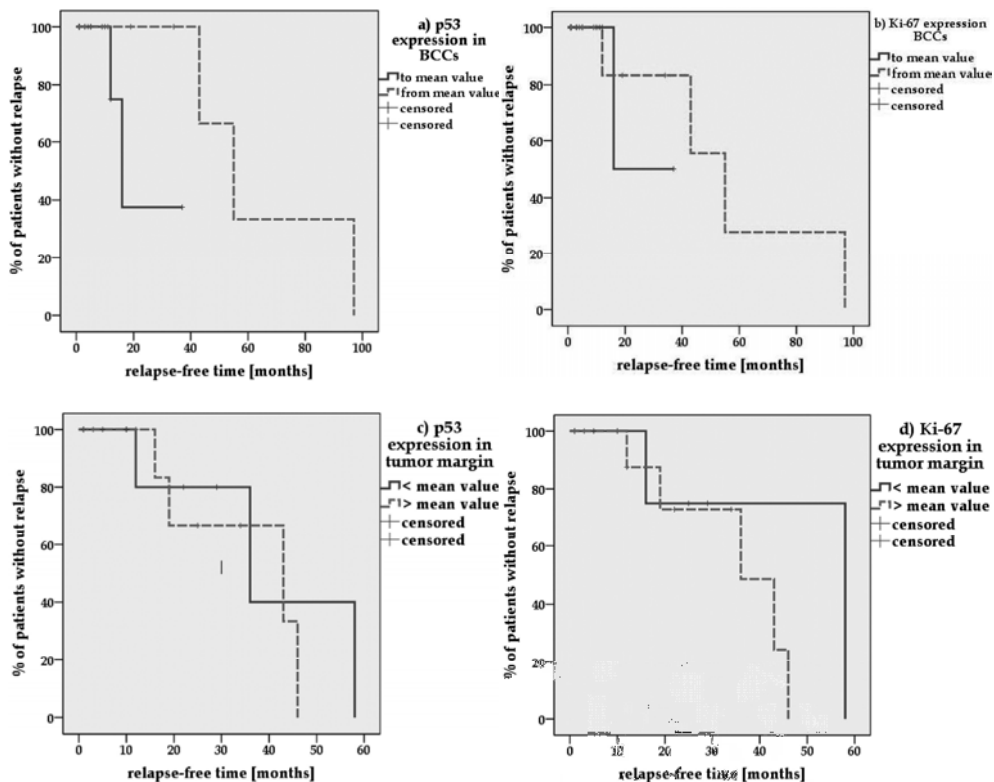


Fig. 7. Kaplan-Meier curves for p53 expression (a, c) and Ki-67 expression (b, d) in BCC tumor center tissues (a, b) and in tumor margin tissues (c, d).

## 2.4 The biology of telomerase

In 1984 Blackburn and Greider discovered telomerase in a single-celled ciliate *Tetrahymena* (165, 166), a ribonucleoprotein enzyme. The enzyme is of universal importance for cell proliferation. Telomerase replaces the mitotic loss of telomeres at chromosome ends with telomeric substitutes. This abrogates the limited cell division potential (the Hayflick limit). Hayflick and Moorhead (167) observed limited cell division in fibroblast cultures. Activation of telomerase occurs during embryogenesis (168, 169). In normal somatic tissues with a limited replicative potential telomerase activity can be demonstrated only in traces or not at all (170 to 172). Activation of telomerase can be found inside cells with proliferative potential such as stem cells, in strongly regenerative cells, and in dermal cells of hair follicles (169, 171 to 175). Telomerase can be activated by UVB in seriously sun-exposed skin (10, 64). It may also be detected in benign proliferative lesions (174) or after activation in cells of inflammatory reactions (170, 172, 174, 176 to 178). Reactivation of telomerase also occurs in carcinogenesis (179, 180), as was illustrated by Ueda et al. (64) (Figure 1, 1.1). It is an essential step for cancer immortalization and cancer progression (172, 180).

However not until Kim et al. (168) developed the polymerase chain reaction based telomeric repeat amplification protocol in 1994 (TRAP assay) was it possible to detect telomerase in greater numbers in unfixed tissues. Since then, a great number of tissues, particularly from cancer, have been investigated for telomerase activity as the reviews by Shay and Bacchetti (181) and Dhaene et al. (182) demonstrate.

#### 2.4.1 Activation of telomerase in tumor center and tumor margin tissues of BCCs

The results of telomerase activity in BCCs and tumor-free adjacent tissues have been summarized. The portion of 203 specimens with evidence of telomerase detected with the TRAP assay (168) amounted to an average of 87%, varying between 20 and 100% (64, 103, 104, 183 to 189). Table 1 sums up telomerase activity of BCCs and tumor-free adjacent tissues. To discriminate between BCC center tissue and tumor-free margin tissue Taylor et al. (183) and Ueda et al. (64) introduced proof of telomerase activity. They found clearly less telomerase activity in tumor margin tissue than in the tumor center: BCC margin tissues with 67% and 39% vs. 95% and 85% of BCC center tissues.

References	telomerase positive in tumor center tissues	telomerase positive in tumor margin tissues
Taylor et al., 1996 (183)	73/77 (95%)	38/57 (67%)
Ueda et al., 1997 (64)	11/13 (85%)	11/28 (39%)
Parris et al., 1999 (184)	10/13 (77%)	
Wu et al., 1999 (185)	10/11 (91%)	
Kim et al., 2000 (186)	1/ 5 (20%)	
Chen et al., 2001 (187)	22/22 (100%)	
Boldrini et al., 2003 (188)	15/20 (75%)	
Fabricsius et al.,2003 (103) and 2006 (104)	26/30 (87%)	9/25 (36%)
Saleh et al., 2007 (189)	9/12 (75%)	
<b>altogether</b>	<b>177/203 (87%)</b>	<b>58/110 (53%)</b>

The extinction values of the PCR-ELISA were negative at 0-150 mOD, 1+ = 150-450 mOD, 2+ = 450-750 mOD 3+ = 750-1050 mOD and 4+ above 1050 mOD (102, 103).

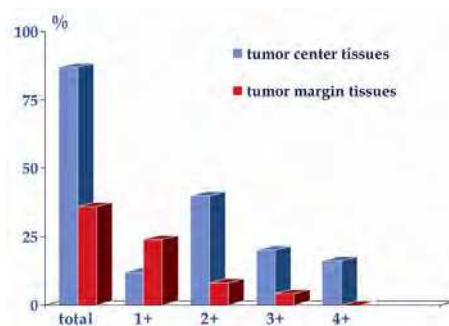


Fig. 8. Telomerase activity in BCCs and in tumor-free margin tissues (103, 104)

Table 1. The frequency of telomerase activity (detected with the TRAP assay) in basal cell carcinomas and their tumor-free margin tissues.

Like Taylor et al. (183) and Ueda et al. (64), we found significantly higher telomerase activity in 26/30 (87%) of BCC tumor center tissues (mean value  $661 \pm 388$  mOD) than in 9/25 (36%) histopathologically tumor-free tumor margin tissues (mean value  $187 \pm 233$  mOD) (103, 104). As we proved telomerase activity semiquantitatively with a PCR-ELISA (Telo TAGGG Telomerase PCR and PCR-ELISA plus, Roche Diagnostics, Germany) we were able to be somewhat more precise: the mean value of telomerase activation in BCC tumor tissues was significantly higher than in tumor margin tissues:  $p < 0.001$ . In contrast, telomerase activation

in T2 tumors (mean value  $564 \pm 225$  mOD) did not differ significantly from telomerase activation in T1 tumors (mean value  $709 \pm 446$  mOD):  $p=0.783$ . In Figure 8, our study on telomerase activation in tumor center and tumor margin has been compiled according to extinction values.

We tested whether the telomerase examined in BCCs or tumor margin tissues has prognostic relevance (103, 104). We divided all measured values of telomerase (PCR-ELISA) and divided the patients into two groups, group 1:  $<$  mean value and group 2:  $\geq$  mean value, and used the patients' up-to-date outcome as documented in our hospital. In Kaplan-Meier curves of tumors and tumor margin tissues, patients with higher telomerase activity suffered recurrence earlier than patients with lower telomerase activity but not significantly: tumor center:  $36 \pm 9$  vs.  $97 \pm 0$  months (Log-rank:  $p=0.100$ ), tumor margin:  $19 \pm 1$  vs.  $42 \pm 6$  months (Log-rank:  $p=0.141$ ): Figure 9.

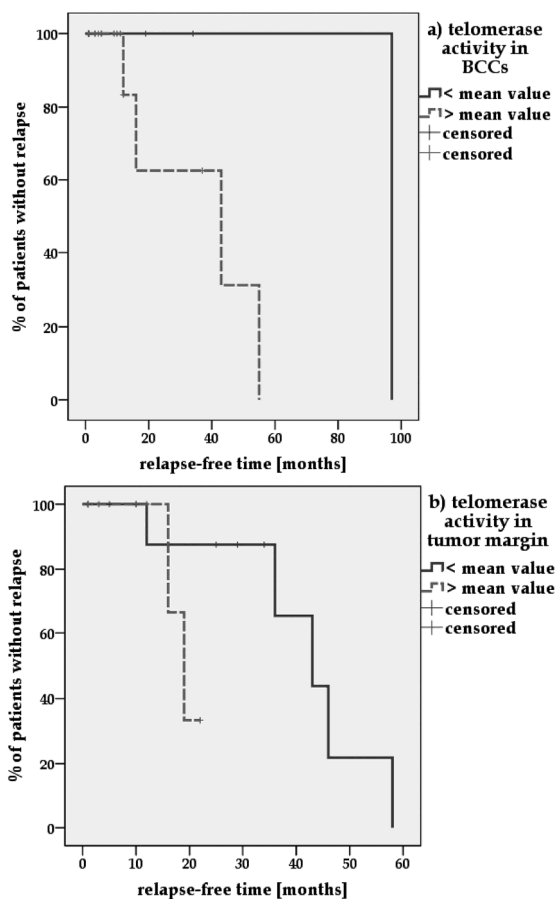


Fig. 9. Comparison of Kaplan-Meier curve for telomerase activity in BCCs (a) and tumor-free margin tissues (b).

Detection of telomerase activity in unfixed BCC tissues and tumor margin tissues can corroborate, with due reservation in view of the censored cases, histopathological and clinical assessment of risk of recurrence.

#### 2.4.2 Expression of hTERT in tumor center and tumor margin tissues of BCCs

hTERT (human telomerase reverse transcriptase) is a catalytic subunit and a component of telomerase (171, 190, 191). Proof of hTERT protein can be carried out both in unfixed and in fixed tissue and facilitates the localization of telomerase activity. The detectable evidence of the hTERT protein or hTERT mRNA might, however, not always mirror detectable telomerase activity (171).

Saleh et al. (189) used proof of hTERT to differentiate between BCCs of differing size and histopathological type. They determined hTERT in all of 12 BCCs, with no significant difference between mRNA hTERT by RTq-PCR in BCCs of different size: mean value BCCs <9 mm 0.458 vs. mean value BCCs ≥9 mm 0.478. In tumor-free margin tissues of larger BCCs the authors (189) found a somewhat lower, non-significant expression of hTERT mRNA (BCC <9 mm mean value 0.160, BCC ≥9 mm 0.119). hTERT expression of superficial BCCs (n=5), however, was significantly lower ( $p < 0.05$ ) than that of nodular BCCs (n=7): tumor center mean value 0.305 vs. 0.525 and tumor-free margin 0.094 vs. 0.172. With senior patients (≥65 years of age, n=6) Saleh et al. (189) succeeded in determining non-significant hTERT expression in tumors and tumor-free margin tissues that was lower than that in younger patients (<65 years of age, n=6): tumor center mean value 0.343 vs. 0.593 and tumor margin 0.104 vs. 0.175. Proof of hTERT was also established by Hu et al. (192) and Park et al. (98) for evaluating BCCs. Hu et al. (192) examined 62 fresh skin tissues in RT-PCR. They compared hTERT with TPI (another protein component of telomerase) and with hTR, an RNA component of telomerase (171). Part of their study (192) was the investigation of 4 BCCs, all of which expressed hTERT, TPI and 3 / 4 hTR. Park et al. (98) demonstrated hTERT 2+ to 3+ in 8 / 10 BCCs in paraffin sections with anti-hTERT antibody from Calbiochem (USA, Ab-2). Ogoshi et al. (193) used in situ hybridization to prove hTERT and confirmed its occurrence in 93% (14/15) of BCCs.

Attia et al. (194) introduced the anti-hTERT antibody code NCL-hTERT (clone 44F12, Novocastra, UK) for evidence of immunohistochemical hTERT in normal tissues such as in photo-exposed skin. The authors succeeded in proving hTERT (1+ to 3+) in 80% of the epidermis of photo-aged subjects. hTERT was expressed in normal skin in the basal layer and in some supra-basal layers, as well as in hair follicles (cf. telomerase activity in hair follicles: 173 to 175).

In our study with small frozen sections we used eleven anti-hTERT antibodies to detect and localize hTERT expression in 25 BCC tumor tissues and 25 tumor margin tissues (107). Previously we had employed the same antibodies to examine squamous cell carcinomas and SCC tumor margin tissues (106). Before performing APAAP (105), slides were fixed in methanol and acetone and pretreated in a steamer (95 to 99°C). As described in detail in our first study (106), this pretreatment was essential for hTERT nuclear staining in frozen sections. The most prominent immunohistochemical effects were achieved with anti-hTERT antibody code NCL-hTERT (clone 44F12, Novocastra, UK),

which was also used by Attia et al. (194) for hTERT detection in sun exposed skin. This antibody was classified later in the paper by Wu et al. (195) as an antibody against nucleolin. We expanded our investigation and incorporated an anti-nucleolin antibody to clarify this issue (106, 107). On the basis of our peptide absorption studies (106) and in agreement with many authors we think that this antibody clone 44F12 Novocastra identifies hTERT. We compared the results of BCCs (107) with antibody Calbiochem, USA, code 582005 and others, and determined the hTERT scores as described in 2.2 and 2.3.1. In the quantitative presentation of antigen expression we refer exclusively to the nuclear and / or nucleolar staining. We evaluated staining intensity (SI) and the number of positive stained cells (PP) in the BCCs and in squamous cell epithelia of the tumor margin. The IRS was calculated by multiplication of SI and PP (144, 145).

Our immunohistochemical proof of hTERT is predominantly localized in tumor and in squamous cell epithelia of BCCs and BCC tumor-free margin tissues. hTERT scores with a polyclonal antibody (Calbiochem/USA, code 582005, Ab 1) and with a monoclonal antibody (Novocastra/UK, code NCL-hTERT, clone 44F12, Ab 2) are significantly lower in the tumor margin (mean score values Ab 1  $5.4 \pm 3.3$  and Ab 2  $7.0 \pm 2.9$ ) than in tumor center (mean score values Ab 1  $9.4 \pm 4.9$  and Ab 2  $9.0 \pm 3.9$ ):  $p=0.002$  and  $p=0.011$  (107). In Figures 10 and 11, hTERT expression is demonstrated with two antibodies. Proof of hTERT expression now and then differs from telomerase activity.

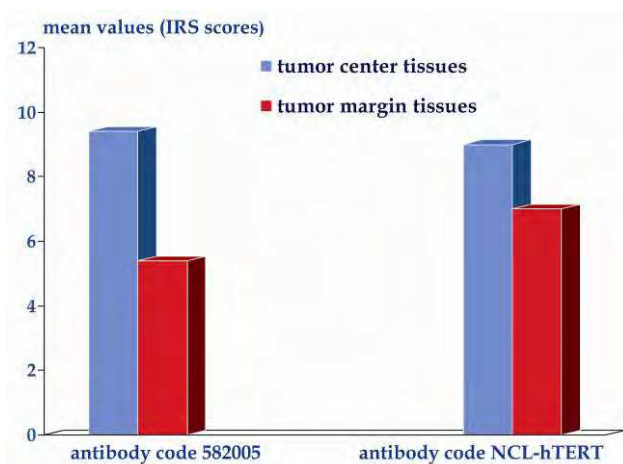
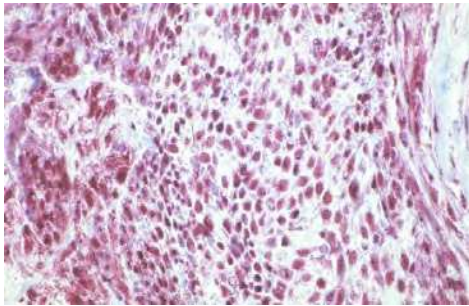


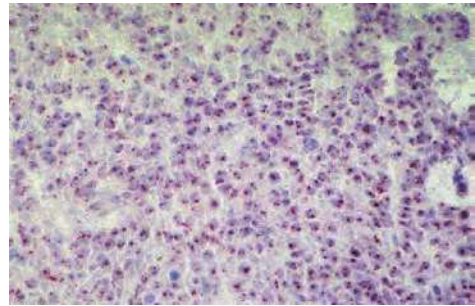
Fig. 10. Mean values of immunoreactive score (cf. 144, 145) with two anti-hTERT antibodies (Calbiochem/USA and Novocastra/UK).



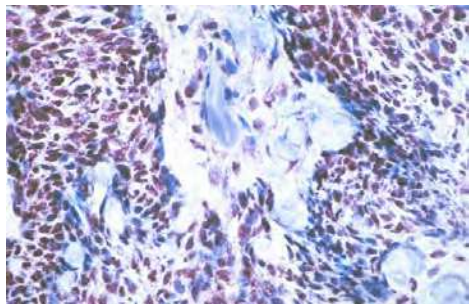
a) tumor center tissue of a nodular basal cell carcinoma (telomerase activity 3+), Ab 1



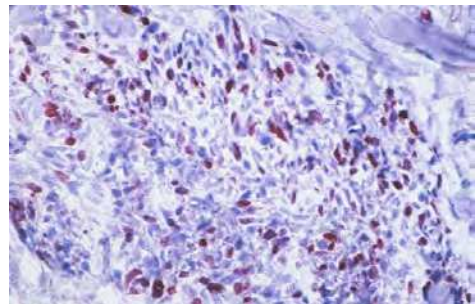
b) tumor center tissue of a nodular basal cell carcinoma (telomerase activity 3+), Ab 2



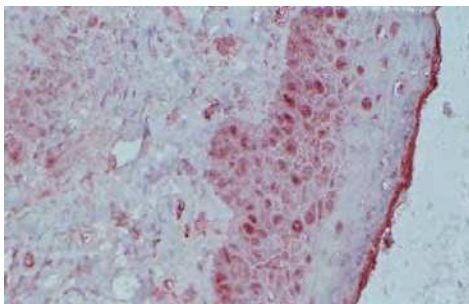
c) tumor center tissue of a mixed nodular-sclerodermiform BCC (telomerase activity 1+), Ab 1



d) tumor center tissue of a mixed nodular-sclerodermiform BCC (telomerase activity 1+), Ab 2



e) tumor margin tissue of a nodular BCC (telomerase activity 1+), Ab 1



f) tumor margin tissue of a nodular BCC (telomerase activity 1+), Ab 2

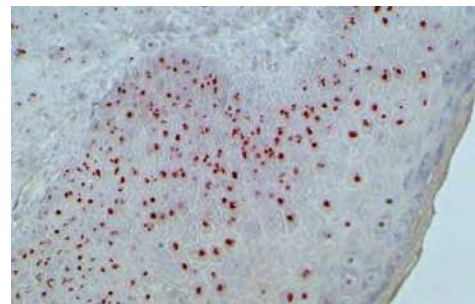


Fig. 11. Comparison of hTERT expression with two anti-hTERT antibodies: polyclonal antibody code 582005, Ab 1 (a, c and e) and monoclonal antibody code NCL-hTERT, clone 44F12, Ab 2 (b, d and f), magnification 400x.

Kaplan-Meier curves indicate that proof of hTERT does not reveal the further course of the disease. We divided all hTERT scores into two groups (group 1: < mean value, group 2:  $\geq$  mean value) and related them with the patients' up-to-date outcome as documented in our hospital. With the caveat that there were many censored cases, it can be said that patients with higher hTERT scores in tumor center tissues relapsed earlier than patients with lower hTERT scores in tumor center tissues: antibody code 582005 (Ab 1):  $43 \pm 8$  months vs.  $70 \pm 31$  months, Log-rank  $p=0.539$ , Figure 12a; antibody code NCL-hTERT (Ab 2):  $42 \pm 9$  months vs.

77±25 months, Log-rank  $p=0.387$ , Figure 12b. However, patients with higher hTERT scores in BCC tumor-free margin tissues did not relapse earlier than those with lower hTERT scores in tumor-free margin tissues: antibody code 582005 (Ab 1): 43±9 months vs. 30±9 months, Log-rank  $p=0.230$ , Figure 12c; antibody code NCL-hTERT (Ab 2): 39±5 months vs. 32±5 months, Log-rank  $p=0.686$ , Figure 12d.

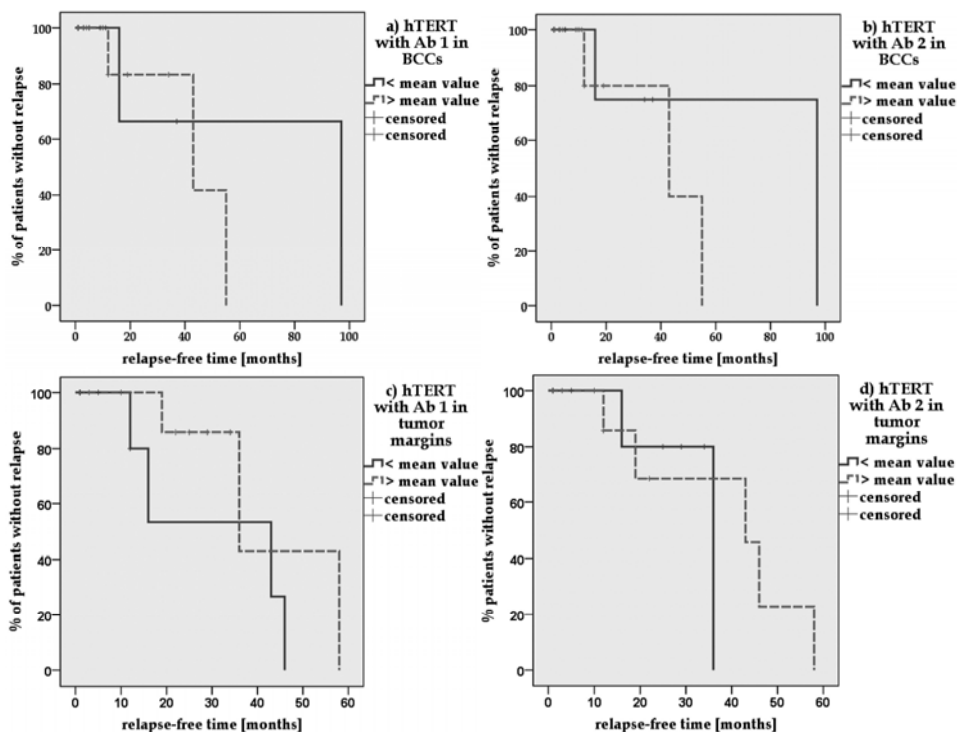


Fig. 12. Kaplan-Meier curves of hTERT expression in BCCs (a and b) and tumor-free margin tissues (c and d): Ab 1 = polyclonal antibody code 582005, Ab 2 = monoclonal antibody code NCL-hTERT.

Our hTERT results in tissues from patients with basal cell carcinoma (107) verify the value of hTERT in localizing the cells in which telomerase is activated (2.4.1). Although we identified differences between eleven antibodies (106, 107), we succeeded in detecting hTERT expression primarily in tumor cells and in squamous epithelia cells (Figure 11) after pretreatment of frozen sections in the steamer. We applied the same unfixed tissues for proof of telomerase activity and for hTERT expression. As McKenzie et al. (171) had already discovered, our detection of hTERT might not always mirror detectable telomerase activity (Figure 11, a to f). Kyo et al. (196) arrived at the same conclusion that hTERT did not always correlate with telomerase activity of the various cell types.

Given our previous observations, we decided to evaluate only nuclear or nucleolar expression of hTERT although some antibodies also displayed cytoplasmic staining (106, 107). It must be mentioned, however, that investigations of gynaecologic carcinomatous tissues by Kyo et al. (196) established proof of both hTERT and high levels of telomerase

after the isolation of cytoplasm, so further evaluation for immunohistochemical proof of hTERT is warranted. Taken together, both telomerase and hTERT may be useful as biomarkers in predicting chemotherapeutic effects (197).

### 3. Biomarkers for chemoprevention of basal cell carcinomas

Given frequent BCC recurrence and field cancerization in tumor-free margin tissues, several studies were launched to investigate the efficacy of chemoprevention in patients with a basal cell carcinoma. The best and primary prevention of basal cell carcinomas is reduction of the most important risk factor, exposure to strong sunlight from childhood (11, 12, 15, 18, 21, 23, 39, 52, 198). The use of agents that inhibit the noxious influence of UV light (199) could therefore be the most effective preventive method. In addition, there have been many studies applying retinoids (topical and oral / systemic) to suppress the emergence of basal cell carcinomas (200 to 207) and thus influence field cancerization of skin and tumor surroundings (59 to 63) to prevent new tumors and / or tumor recurrences. For immunosuppressed patients, chemoprevention is particularly important (63, 198, 208 to 210). After chemoprevention proved to be unsuccessful in most studies, receptor-selective retinoids such as tazarotene were tried out (211 to 218). Tazarotene is selective for retinoic acid receptors RAR $\beta$  and RAR $\gamma$ . Thus Bianchi et al. (217) successfully applied tazarotene-gel preoperatively for small superficial and nodular BCCs. This was not successful on keratotic BCCs with high p53 expression, and they were operated afterwards. Crucial for effective retinoic acid chemoprevention and / or therapy is the expression of RAR- and RXR-receptors (218, 219), as was demonstrated by So et al. (220). So et al. consider tazarotene to be the most appropriate agent to date for chemoprevention of BCCs.

In addition to synthesis of suitable agents it is essential to search for suitable biomarkers to improve prevention (19), and markers such as p53, Ki-67 or telomerase (2.3 and 2.4) merit consideration along with other genetic markers. Intermediate markers are needed in particular for chemoprevention trials (204). To determine a preliminary endpoint, genetic, cellular, biochemical and immunological surrogate biomarkers - surrogate endpoint markers (SEBs) - are being used and validated so that BCC chemoprevention can be evaluated before a recurrence becomes evident (204, 221 to 225). Orlandi et al. (225) employed the markers Ki-67, p53, apoptosis-index, RAR $\alpha$ , RAR $\beta$  and RAR $\gamma$  before and after tazarotene therapy of 30 BCCs. Expression of Ki-67 was significantly reduced after therapy compared with controls (11% vs. 29%,  $p < 0.001$ ). Expression of p53 was unchanged after therapy. In comparison with controls, however, evidence of apoptotic cells was significantly higher after therapy (4.5% vs. 1%,  $p < 0.001$ ). While expression of RAR $\alpha$  and RAR $\gamma$  was not changed through therapy, expression of receptor RAR $\beta$  was distinctly higher. In our model investigations with cell cultures of oral squamous cell carcinomas, telomerase proved to be a suitable biomarker before and after retinoid therapy (162). Verification with BCCs is yet to follow.

Examination of RAR receptors (retinoic acid receptors) and RXR receptors (retinoid X receptors) on BCCs might indicate why most studies do not show any differences between therapy and placebo groups. Using immunohistochemical methods, Kamradt and Reichrath (226) investigated expression of RAR receptors in frozen sections of 15 basal cell carcinomas: RAR $\alpha$  was most evident (3 +), RAR $\gamma$  a little weaker (2 +) and RAR $\beta$  scanty (0 to 1 +). This corroborates studies by Hartmann et al. (227). In comparison with SCCs of the skin, the authors determined RAR receptors of 28 BCCs by RTq-PCR. Measured values of this study

were also lowest for RAR $\beta$ -mRNA ( $7.91 \times 10^{-5}$ ) and distinctly higher for RAR $\alpha$  ( $1.97 \times 10^{-3}$ ) and RAR $\gamma$  ( $1.25 \times 10^{-2}$ ).

Approximately comparable with regard to tumor-free margin tissues are immunohistochemical investigations by Reichrath et al. (228) on normal skin RAR- and RXR receptors of 12 male volunteers. Expression of RAR $\alpha$  was most distinctly detectable, among other things, in epidermal keratinocytes (5+) and in hair follicle keratinocytes (5+). Expression of RAR $\gamma$  was distinct in epidermal keratinocytes (2+) and in hair follicles (3+), but expression of RAR $\beta$  was found in traces only (0 to 1+). All three RXR receptors RXR $\alpha$ , RXR $\beta$  and RXR $\gamma$  were distinctly expressed (2+, mostly 3+) in both of these cell types.

We therefore tested as well for expression of RAR and RXR receptors in small frozen sections of BCCs with immunohistochemical APAAP (105). After fixing pretreatment in a steamer, which was essential in our study for clear and strong nuclear receptor detection in frozen sections (162, cf. 106, 107), we applied antibodies from Santa Cruz / USA (anti-RAR $\alpha$ , code sc-551; anti-RXR $\beta$ 1, code sc-556 and anti-RXR $\gamma$ , code sc-555), from Abcam / USA (anti-RAR $\beta$ , code ab15515) and from Abnova / USA (anti-RXR $\alpha$ , code H00006256 M01). Other antibodies were less suitable for our frozen sections. In our study the anti-RAR $\gamma$  antibody was inappropriate (162). Evaluation succeeded with immunoreactive scores (IRS) as described with p53, Ki-67 and hTERT (2.3.1, 2.3.2 and 2.4.2). For control we investigated tissues with comparable localization from BCC-free patients. Figure 13 summarizes mean score values for

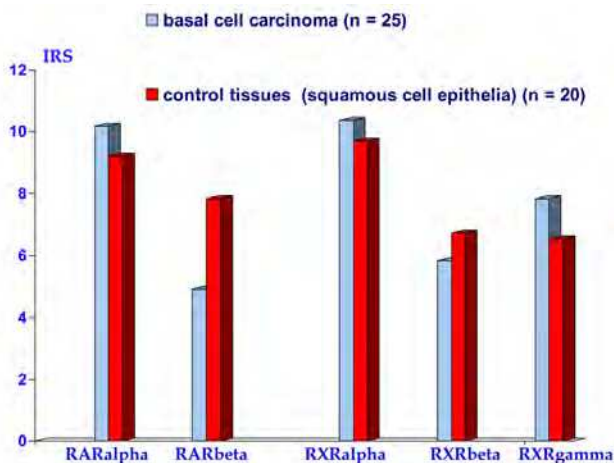


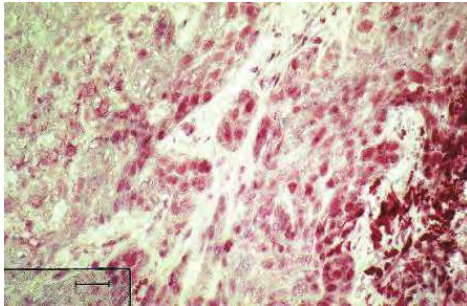
Fig. 13. Immunoreactive scores (IRS, cf. 144, 145) of expression for RAR and RXR receptors in small frozen sections of BCCs and control tissues (mean values).

expression of RAR and RXR receptors. In agreement with Kamradt and Reichrath (226) and with Hartmann (227) we were able to detect all receptors tested in BCCs in similar levels as in control tissues. RAR $\beta$  expression in BCCs had the lowest value (mean score value  $4.92 \pm 1.81$ ), followed by RXR $\beta$  (mean score value  $5.83 \pm 1.76$ ), RXR $\gamma$  (mean score value  $7.83 \pm 2.36$ ), RAR $\alpha$  (mean score value  $10.17 \pm 2.02$ ) and RXR $\alpha$  (mean score value  $10.38 \pm 2.14$ ). Expression of RAR $\beta$  was almost significantly lower in BCCs than in control tissues ( $p=0.056$ ). The expression of the

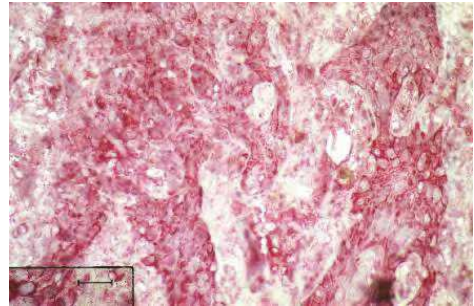
remaining receptors in BCCs and control tissues did not differ statistically:  $p=0.586$  ( $RAR\alpha$ ),  $p=0.862$  ( $RXR\alpha$ ),  $p=0.863$  ( $RXR\beta$ ) and  $p=0.871$  ( $RXR\gamma$ ).

Our results suggest that the increase of  $RAR\beta$  in the context of a chemoprevention might give us valuable information as a surrogate end point biomarker for the success of therapy in chemoprevention, as was previously demonstrated by Orlandi et al. (225). Figures 14a to 14e illustrate expression patterns of receptors in small frozen sections of basal cell carcinomas.

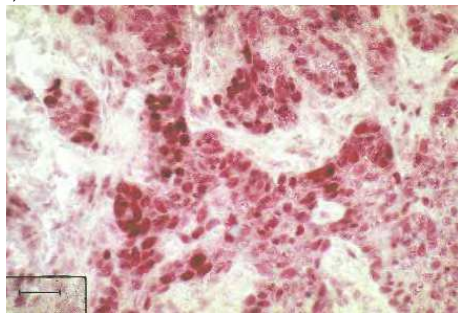
a)  $RAR\alpha$



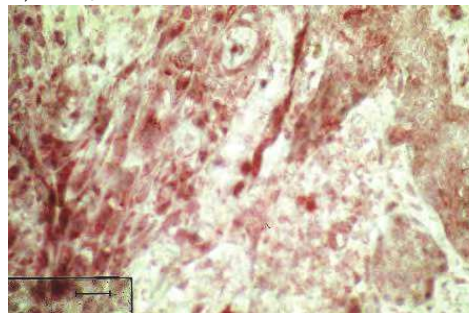
b)  $RAR\beta$



c)  $RXR\alpha$



d)  $RXR\beta$



e)  $RXR\gamma$

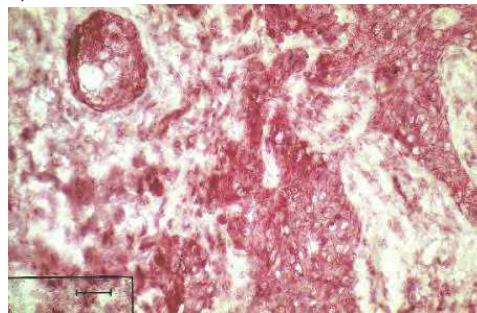


Fig. 14. Expression of retinoic acid receptors (RAR) and retinoid X receptors (RXR) of a nodular BCC (frozen sections); bars 20  $\mu\text{m}$ .

#### 4. Conclusion

Immunohistochemical application of Ber-Ep4 (85 to 88) is particularly appropriate for histopathological differentiation between SCCs of the skin and basal cell carcinomas. When examined with immunohistochemical methods, adhesion molecules CD44v6 and U36 (CD44v6-chimera) give clear evidence for spreading of BCCs (Figure 4) and for the absence of tumor in tumor margins (91 to 96, 134 to 138, cf. 131 to 133). Radionuclide-labeled antibodies developed against oral squamous cell carcinomas (146 to 156, 229, 230) might also be applied in the diagnosis and therapy of patients with BCC.

It is important for prognostic purposes to make a clear distinction between smaller T1-BCCs and larger T2-BCCs (cf. 2. and Figure 2). Clinical classification into aggressive and less aggressive BCCs confirmed by histopathology can be supported, among other things, by markers p53 (101, 103, 121 to 124, 157 to 161), bcl-2 (81, 100, 122), Ki-67 (100, 106, 107, 158, 161) and by the apoptotic index (124). The markers Ki-67 and p53 can impart supplementary information and are important for histopathological diagnosis with regard to making a clinical judgement of the consequences of "tumor-free margin". Representation of telomerase activity in unfixed tumor margin tissues (64, 103, 104, 183), and not so much immunohistochemical proof of hTERT, (98, 107, 189, 192) can contribute to a better diagnostic assessment of tumor-free margins and may indicate early recurrence.

The high proportion of BCC recurrences is a challenge to develop better selective agents for chemoprevention and to support them with appropriate biomarkers such as Ki-67, apoptotic index, telomerase or by RAR $\beta$  (199, 214, 225, cf. 162).

#### 5. Acknowledgements

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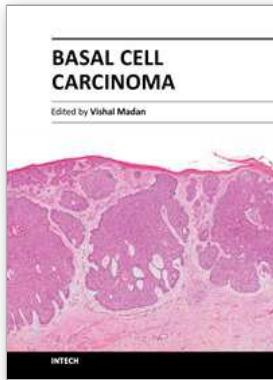


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Basal cell carcinoma is the commonest cutaneous malignancy. The last decade has witnessed exponential research which has broadened our understanding of the pathogenesis of basal cell carcinomas. This is also important from a therapeutic point of view as targeted approach to therapy is now being increasingly experimented. Although it is impossible to condense and present all good research in one book, the authors have to be commended on presenting their research on several aspects of basal cell carcinoma in a succinct manner, which shall not only enhance our understanding of, but also hopefully via this open exchange of ideas pave ways for successful targeted therapy of the commonest human cancer.

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