***FGFR3* mutations in seborrheic keratoses are already present in flat lesions and associated with age and localization**

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Somatic activating fibroblast growth factor 3 (*FGFR3*) mutations in human skin can cause seborrheic keratoses, one of the most frequent skin tumors in man. However, details of the involved mechanisms remain elusive. We analyzed 65 acanthotic seborrheic keratoses with varying vertical diameters for *FGFR3* mutations using a SNaPshot[®] multiplex assay. Immunohistochemistry was performed for Ki-67, bcl-2 and *FGFR3* protein in all seborrheic keratoses and 19 normal skin samples. *FGFR3* mutations were detected in 37 of 65 seborrheic keratoses (57%). These mutations were found both in flat (initial) and thick seborrheic keratoses. *FGFR3* mutations were significantly associated with increased age and localization on the head and neck ($P < 0.01$). Ki-67 expression was significantly higher in seborrheic keratoses than in normal epidermis independent of the *FGFR3* status ($P < 0.001$). Furthermore, *FGFR3* mutations were associated with an increased expression of bcl-2 and *FGFR3* protein ($P < 0.05$). Our results indicate that *FGFR3* mutations can occur early in the pathogenesis of at least a subset of seborrheic keratoses. Increased age appears to be a risk factor for these mutations. The preferential occurrence of *FGFR3* mutations in seborrheic keratoses of the head and neck suggests a causative role for cumulative lifetime ultraviolet light exposure.

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Seborrheic keratoses represent one of the most common skin tumors in man. Their prevalence increases with age. Seborrheic keratoses are detected in 80–100% of people over 50 years.^{1,2} Affected individuals can show large numbers of lesions. Yeatman *et al* reported an average number of 69 seborrheic keratoses/person in people aged more than 75 years. Seborrheic keratoses are typically localized on the head, the trunk and the extremities with the exception of the palms and soles. They present as well-demarcated brownish plaques and later may show a verrucous surface. Their horizontal diameter ranges from a few millimeters to several centimeters. Three major histologic subtypes are known including acanthotic, hyperkeratotic and

adenoid seborrheic keratoses variants.³ Common histological features are acanthosis, papillomatosis and hyperkeratosis along with a varying degree of pigmentation. The vertical diameter (tumor thickness) of seborrheic keratoses varies considerably. Flat (initial) seborrheic keratoses frequently show gradual vertical growth within years.³ However, malignant transformation is a very rare event in seborrheic keratoses, and the reported cases may also represent collision tumors of seborrheic keratoses and other epidermal malignancies such as basal cell carcinoma and squamous cell carcinoma.⁴

Although seborrheic keratoses are very common tumors and represent one of the most disfiguring signs of skin aging, their pathogenesis is only marginally understood. Recently, activating *FGFR3* mutations in the epidermis were shown to be involved in the development of seborrheic keratoses.⁵ Transgenic mice expressing the S249C *FGFR3* mutation in the basal layer of the epidermis under the control of the keratin 5 promoter developed thickening of the skin and verrucous skin tumors

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with histological features similar to human seborrheic keratoses. *FGFR3* mutations have been identified in 39–85% of human seborrheic keratoses.^{5,6} In patients with *FGFR3* mutant seborrheic keratoses, normal skin showed a wild-type *FGFR3* sequence confirming the somatic nature of these mutations. Albeit an important role of *FGFR3* mutations in the pathogenesis of seborrheic keratoses is suggested by previous studies, details of the mechanisms causing these mutations in human epidermis and of the signaling pathways mediating the growth of acanthotic tumors in *FGFR3* mutant skin are unknown. Therefore, we analyzed 65 acanthotic seborrheic keratoses for the presence of *FGFR3* mutations. The *FGFR3* status was correlated with the thickness of the seborrheic keratoses to determine whether *FGFR3* mutations are early or late genetic alterations in the pathogenesis of seborrheic keratoses. In addition, the *FGFR3* status was correlated with Ki-67, bcl-2 and *FGFR3* protein expression to investigate whether increased proliferation, anti-apoptotic mechanisms and *FGFR3* protein overexpression are associated with *FGFR3* mutations in human seborrheic keratoses.

Materials and methods

Patient Material and Microdissection

Sixty-five histologically confirmed acanthotic seborrheic keratoses of 65 individuals (33 men and 32 women) were retrieved from the histology files of the Department of Dermatology, University of Regensburg. The characteristics of the persons are shown in Table 1. Nineteen normal skin samples from resection margins served as controls for immunohistochemical analyses. From two patients with seborrheic keratoses, normal skin adjacent to the seborrheic keratoses was additionally available. Informed consent had been obtained from all patients according to the guidelines of the local ethics committee and the declaration of Helsinki. The vertical diameter (thickness) of each acanthotic seborrheic keratosis was measured on an H&E stained reference section using a scale bar. Furthermore, sections of 10 μ m thickness from formalin-fixed and paraffin-embedded seborrheic keratoses tissues were microdissected manually with a needle under an inverted microscope to obtain at least 80% seborrheic keratoses cells.

DNA Isolation

Isolation of DNA was performed following standard protocols. In brief, about 10–50 mg of dissected tissue was digested with proteinase K overnight in lysis buffer, and DNA was isolated with the Magna Pure[®] PCR Template Preparation Kit (Roche Diagnostics) according to the manufacturer's protocol.

SNaPshot[®] Multiplex Assay

We used a previously described high-throughput SNaPshot multiplex mutation detection assay for the screening of 11 activating *FGFR3* point mutations.^{7,8} In brief, three regions of interest in exons 7, 10 and 15 comprising known *FGFR3* point mutations were amplified in one multiplex polymerase chain reaction. Mutation-specific primers were then extended with labeled di-deoxynucleotides. The SNaPshot multiplex assay covers the following 11 *FGFR3* point mutations: c.742C>T (R248C), c.746C>G (S249C), c.1114G>T (G372C), c.1117A>T (S373C), c.1124A>G (Y375C), c.1144G>A (G382R), c.1178C>A (A393E), c.1954A>G (K652E), c.1954A>C (K652Q), c.1955A>T (K652M), c.1955A>C (K652T). The nucleotide positions correspond to the coding sequence of the *FGFR3* IIIb isoform, where position 1 is the A of the ATG. *FGFR3* IIIb is the predominantly expressed isoform in epithelial cells such as keratinocytes. The assay covered all yet-identified *FGFR3* mutations in benign acanthotic skin tumors (seborrheic keratoses, epidermal nevi) and urothelial carcinoma. The presence or absence of a mutation was indicated by the incorporated wild type or mutant labeled di-deoxynucleotide. Each mutation was confirmed by an independent reaction.

Immunohistochemistry

Immunohistochemical analysis was performed in 65 acanthotic seborrheic keratoses and 19 normal skin samples for the analysis of Ki-67, bcl-2 and *FGFR3* protein expression. Sections of paraffin-embedded tissues with a thickness of 1.5 μ m were deparaffinized, rehydrated and blocked with 3% H₂O₂/70% CH₃OH for 10 min. They were incubated in citrate buffer (pH 6.0) at 90°C for 20 min. Then the samples were incubated with the primary antibody dilution at room temperature for one hour (Ki-67 1:100, bcl-2 1:50, *FGFR3* 1:50). Antibodies were purchased from Dako (Ki-67, bcl-2) and Santa Cruz (*FGFR3*). Staining was performed using the Zyto Chem Plus HRP Broad Spectrum Kit (Zytomed, Berlin) according to the manufacturer's protocol. Negative controls were performed by omitting the primary antibody. Evaluation of the protein expression was performed without knowledge of the *FGFR3* mutational status of the samples. Ki-67-positive cells were counted in five representative areas of each section, and at least 200 nuclei were analyzed in each of these areas. The percentage of Ki-67-positive cells was then calculated for each section. Immunohistochemical staining for bcl-2 and *FGFR3* was evaluated using a semiquantitative score: 0 (not expressed), 1 (positive staining of about 1–30% of cells), 2 (positive staining of about 31–60% of cells) and 3 (positive staining of more than 60% of cells).

Table 1 Analysis of 65 acanthotic seborrheic keratoses

No.	Sex	Age	Localization	Thickness (mm)	FGFR3 mutation analysis	Immunohistochemistry		
						Ki-67 (%)	bcl-2	FGFR3
1	m	75	Trunk	0.13	wt	4.8	++	++
2	m	41	Head	0.15	wt	3.4	++	+
3	m	45	Head	0.28	K652M	6.9	++	++
4	f	72	Trunk	0.42	wt	3.4	+	+
5	f	57	Trunk	0.36	S373C	11.6	+	+
6	m	64	Head	0.28	R248C	5.2	+++	+
7	f	64	Trunk	0.10	S249C	3.0	++	+
8	m	49	Trunk	0.25	wt	4.0	+	+
9	f	67	Arm	0.35	wt	8.5	+	++
10	f	48	Trunk	0.72	wt	18.1	+++	++
11	f	56	Trunk	0.10	wt	6.4	+	+
12	f	52	Neck	0.43	K652M	3.5	++	+
13	m	59	Head	0.37	Y375C	5.1	++	+
14	f	51	Trunk	0.13	wt	2.5	++	++
15	f	70	Neck	1.35	K652M	3.4	+++	++
16	m	63	Trunk	0.10	wt	3.5	+	+
17	f	61	Trunk	1.30	K652M	10.7	++	++
18	m	61	Head	1.10	G372C	9.5	+++	++
19	f	49	Trunk	1.00	Y375C	7.6	++	++
20	m	78	Trunk	0.47	K652M	3.0	++	++
21	m	78	Head	0.55	wt	6.0	++	+
22	f	74	Trunk	1.00	K652E	7.9	+	+
23	f	67	Trunk	1.10	wt	8.1	+	+
24	m	56	Head	1.05	S249C	9.4	++	++
25	m	64	Trunk	0.85	K652E	5.9	+	+
26	f	84	Head	1.30	K652M	3.4	+++	++
27	m	68	Trunk	1.20	K652M	4.3	++	++
28	m	60	Neck	1.30	wt	1.8	++	+++
29	m	65	Trunk	0.52	R248C	8.9	+++	++
30	f	52	Trunk	1.20	K652M	1.5	+	++
31	f	82	Head	0.65	R248C	6.4	+	++
32	m	75	Trunk	0.38	wt	5.0	+++	++
33	m	67	Head	1.30	R248C	14.9	+	++
34	f	66	Thigh	0.90	wt	10.2	++	++
35	f	67	Neck	0.72	Y375C	3.8	+++	++
36	m	62	Head	1.10	R248C	4.6	+	+
37	f	71	Head	0.94	R248C	5.1	++	++
38	m	44	Trunk	1.00	wt	5.4	++	++
39	f	55	Neck	2.05	G372C	7.4	+	++
40	m	66	Head	0.90	S249C	6.5	+++	+++
41	m	63	Trunk	0.70	wt	6.5	+	+
42	m	77	Trunk	0.60	wt	3.2	+	+
43	f	66	Head	0.80	R248C	2.8	++	++
44	f	34	Trunk	0.65	wt	11.0	+	+++
45	m	70	Trunk	0.50	Y375C	6.6	—	+
46	m	67	Head	1.25	R248C	12.5	++	+++
47	f	44	Head	0.20	wt	10.5	+	+
48	f	70	Head	1.30	R248C	14.2	++	+++
49	m	39	Trunk	0.80	wt	8.8	+	+
50	m	37	Head	3.20	S373C	8.8	++	+++
51	f	82	Trunk	0.40	R248C	4.5	++	+
52	f	83	Head	0.70	R248C	6.5	++	+++
53	f	26	Trunk	0.70	wt	11.5	+	++
54	f	59	Trunk	0.75	wt	7.2	+++	+++
55	f	51	Trunk	1.35	R248C	6.4	+++	+++
56	m	45	Trunk	2.40	wt	12.7	+	+
57	m	25	Trunk	1.20	wt	12.0	+	+
58	f	59	Trunk	1.60	wt	14.1	+	+
59	m	50	Trunk	1.40	wt	4.3	++	+
60	f	60	Head	1.10	wt	10.0	++	+
61	m	59	Trunk	1.20	R248C	9.2	+++	+++
62	m	63	Head	1.10	R248C	7.8	+	+
63	f	76	Head	0.20	K652M	4.9	++	++
64	m	33	Neck	0.30	wt	11.3	+	+++
65	m	64	Trunk	0.70	G372C	9.1	++	+++

m, male; f, female; age, age at the time of the seborrheic keratoses removal; maximal thickness of acanthotic seborrheic keratoses was measured using a scale bar on an H&E-stained section of each sample; *FGFR3* mutation analysis was performed with a multiplex SNaPshot[®] assay covering 11 known activating *FGFR3* mutations (R248C, S249C, G372C, S373C, Y375C, G382R, A393E, K652E, K652M, K652Q and K652T; codons are numbered according to the open reading frame of the *FGFR3b* isoform, which is predominantly found in epithelial cells); Ki-67 expression was assessed immunohistochemically by counting the proportion of Ki-67-positive cells in five representative areas (at least 200 cells/area) of each section; bcl-2 and *FGFR3* protein expression were detected immunohistochemically and scored from (–) = negative to (+++) = strong positive.

Statistical Analysis

The non-parametric Mann–Whitney *U*-test was used for statistical analysis. In addition, the cross-tab test was used for the analysis of the association between *FGFR3* status and the localization of seborrheic keratoses. A *P*-value <0.05 was considered significant.

Results

Analysis of *FGFR3* Mutations in Acanthotic Seborrheic Keratoses

FGFR3 mutations were found in 37 of 65 seborrheic keratoses (57%), while 28 of 65 seborrheic keratoses

Figure 1 SNaPshot analysis of human seborrheic keratoses. (a) The R248C mutation was the most frequent *FGFR3* mutation detected in this study (patient no. 55). (b) Normal epidermis adjacent to the seborrheic keratoses in patient no. 45 showed a wild-type *FGFR3* status, confirming the somatic nature of *FGFR3* mutations. (c) The seborrheic keratoses of patient no. 45 revealed a Y375C mutation.

(43%) revealed a wild-type status at the investigated loci (Table 1). The most frequent mutation was R248C (Figure 1a). This mutation was detected in 14 of 37 seborrheic keratoses (38%). The numbers of seborrheic keratoses revealing other *FGFR3* mutations were *n*=9 (24%) for K652M, *n*=4 (11%) for Y375C, *n*=3 (8%) for S249C, *n*=3 (8%) for G372C, *n*=2 (5%) for S373C and *n*=2 (5%) for K652E (Table 2). Neither the A393E mutation nor double mutations, which both had been described previously in seborrheic keratoses,⁶ were observed in this study. From two individuals (no. 44 and 45), additional normal skin adjacent to the seborrheic keratoses was available for mutation analysis. Both normal skin samples displayed a wild-type *FGFR3* sequence (Figure 1b), while the seborrheic keratoses of patient 45 showed a Y375C mutation (Figure 1c). This finding confirms the somatic nature of *FGFR3* mutations in human seborrheic keratoses.

FGFR3 Mutations are already Present in Flat Seborrheic Keratoses

The thickness of acanthotic seborrheic keratoses varies considerably. Dermatologists clinically observe flat lesions with a mild acanthosis in histopathology as well as thick and verrucous seborrheic keratoses, histologically showing a marked acanthosis. The flat lesions are often referred to as initial seborrheic keratoses, which can subsequently proceed to thick seborrheic keratoses.³ We sought to investigate whether *FGFR3* mutations are already present in flat (initial) seborrheic keratoses and therefore may represent early genetic alterations in the pathogenesis of seborrheic keratoses, or whether *FGFR3* mutations are associated with more prominent acanthosis. The latter would indicate that these mutations occur later in the pathogenesis of seborrheic keratoses and promote vertical growth of the flat seborrheic keratoses.

To test these two hypotheses, we measured the thickness of the acanthotic seborrheic keratoses in our series (Figure 2a). The mean thickness of all seborrheic keratoses was 0.84 ± 0.56 mm (range: 0.10–3.20 mm). Acanthotic seborrheic keratoses showing a wild-type sequence in the SNaPshot

Table 2 *FGFR3* mutations in 65 acanthotic seborrheic keratoses

<i>FGFR3</i> mutation	Number of SK	% of mutations	Nucleotide position	Predicted effect	Germline ^a
R248C	14	37.8	c.742C>T	p.Arg248Cys	TD I
K652M	9	24.3	c.1955A>T	p.Lys652Met	SADDAN
Y375C	4	10.8	c.1124A>G	p.Tyr375Cys	TD I
S249C	3	8.1	c.746C>G	p.Ser249Cys	TD I
G372C	3	8.1	c.1114G>T	p.Gly372Cys	TD I
S373C	2	5.4	c.1117A>T	p.Ser373Cys	TD I
K652E	2	5.4	c.1954A>G	p.Lys652Glu	TD II

SK, seborrheic keratosis.

^aAssociated phenotype of the mutation in germline (TD I, thanatophoric dysplasia type I; TD II, thanatophoric dysplasia type II; SADDAN, severe achondroplasia with developmental delay and acanthosis nigricans).

Figure 2 Immunohistochemical analysis of acanthotic seborrheic keratoses. (a) The vertical diameter of the acanthotic seborrheic keratoses was measured on H&E stained sections (patient no. 45). (b) Immunohistochemical staining for Ki-67 antigen revealed an increased proliferation rate in seborrheic keratoses compared with normal epidermis (patient no. 60). (c) Immunohistochemical staining for bcl-2 antigen in seborrheic keratoses was significantly higher in *FGFR3* mutant seborrheic keratoses (patient no. 15). (d) Immunohistochemical staining for *FGFR3* protein showed *FGFR3* protein expression in all seborrheic keratoses with a preferential staining of the basal epidermis in most samples (patient no. 31).

analysis showed a mean thickness of 0.71 ± 0.53 mm (Figure 3). The mean thickness of seborrheic keratoses with *FGFR3* mutations was 0.93 ± 0.56 mm. Although *FGFR3* mutant seborrheic keratoses showed a slightly increased mean thickness compared with wild-type seborrheic keratoses, the difference between wild type and mutant seborrheic keratoses was not significant ($P=0.07$). Our results indicate that *FGFR3* mutations occur both in flat and thick seborrheic keratoses. Therefore, *FGFR3* mutations are early genetic events in the pathogenesis of at least a subset of seborrheic keratoses.

***FGFR3* Mutations in Human Seborrheic Keratoses are Associated with Age and Localization**

The age of the analyzed patients ranged from 25 to 84 years (mean age: 60.1 ± 13.6 years; median age: 63

years). Individuals with *FGFR3* mutant seborrheic keratoses were significantly older than individuals with wild-type seborrheic keratoses (Figure 4). The mean age of individuals with wild-type seborrheic keratoses was 54.5 ± 15.0 years, while the mean age of the individuals with *FGFR3* mutant seborrheic keratoses was 64.4 ± 10.5 years ($P=0.009$). Our results suggest that the frequency of the investigated *FGFR3* mutations in human seborrheic keratoses may increase with age. By contrast, there was no significant correlation between the sex and *FGFR3* mutations in seborrheic keratoses ($P=0.70$).

The analyzed seborrheic keratoses of this study were localized on the head (22), neck (6), trunk (35), arm (1) and thigh (1). We investigated whether the *FGFR3* status of seborrheic keratoses is different in chronically UV-light exposed skin (head and neck) versus in skin with intermittent UV-light exposure (trunk, arm and thigh) (Figure 5). Interestingly, 18

Figure 3 The average thickness was not significantly different in *FGFR3* wild-type and mutant seborrheic keratoses, indicating that *FGFR3* mutations occur both in flat and thick seborrheic keratoses. Thus, *FGFR3* mutations appear to be early genetic events in the pathogenesis of at least a subset of seborrheic keratoses (mean values and 95% confidence intervals of the mean values are indicated).

Figure 4 The mean age of individuals with *FGFR3* mutant seborrheic keratoses was significantly higher than that of individuals with wild-type seborrheic keratoses.

of 22 seborrheic keratoses (82%) localized on the head revealed an *FGFR3* mutation, while 15 of 35 seborrheic keratoses (43%) of the trunk showed a mutant *FGFR3*. Seborrheic keratoses of the neck harboured *FGFR3* mutations in four of six cases (67%). Seborrheic keratoses of the head and neck revealed significantly more *FGFR3* mutations than seborrheic keratoses of the trunk and the extremities

Figure 5 Seborrheic keratoses localized on the head and neck showed a significant higher frequency of *FGFR3* mutations than seborrheic keratoses on the trunk and extremities.

($P = 0.003$). The cumulative life-time UV-light exposure of the head and neck region is assumed to be higher than that of the trunk, suggesting that the increased rate of *FGFR3* mutations at these regions may be linked to UV-light exposure. The R248C mutation, the most frequent mutation in this study, represents a typical UV-signature mutation with a C→T transition at a dipyrimidine site. Accordingly, the proportion of seborrheic keratoses of the head and neck region revealing a R248C mutation was 46% (10 of 22 seborrheic keratoses) and therefore higher than the proportion of this mutation in seborrheic keratoses of the trunk and extremities (4 of 15 seborrheic keratoses; 27%), but this was not significant ($P = 0.25$).

Seborrheic Keratoses Show Increased Ki-67 Expression Independent of *FGFR3* Status

The mechanisms leading to the development of acanthosis, papillomatosis, hyperkeratosis and hyperpigmentation in *FGFR3* mutant skin and details of the involved signaling pathways downstream of the mutant *FGFR3* protein in the epidermis are largely unknown. We performed immunohistochemistry for Ki-67 protein to assess a possible contribution of hyperproliferation to the development of human seborrheic keratoses and its association with *FGFR3* mutations (Figure 2b). We additionally analyzed 19 normal skin samples to compare the protein expression of seborrheic keratoses and normal epidermis.

In *FGFR3* wild-type seborrheic keratoses, $7.7 \pm 4.0\%$ of the cells were Ki-67 positive, and in *FGFR3* mutant seborrheic keratoses $6.8 \pm 3.1\%$ (Figure 6). Ki-67 expression as a marker for proliferation was not significantly different in *FGFR3* wild-type and mutant seborrheic keratoses ($P = 0.53$). However, the mean Ki-67 expression in normal epidermis was $2.3 \pm 0.8\%$ and therefore significantly lower than in

Figure 6 Ki-67 expression as a marker of proliferation was significantly increased in seborrheic keratoses compared with normal epidermis independent of their *FGFR3* status.

seborrheic keratoses ($P < 0.001$). These results indicate that Ki-67 expression is increased in the epidermis of human seborrheic keratoses compared with normal epidermis independent of the *FGFR3* status. Remarkably, both seborrheic keratoses groups (wild type and mutant) showed a considerable variance regarding Ki-67 expression (range: 1.5–18%).

FGFR3 Mutations are Associated with Bcl-2 Protein Expression

Resistance to apoptosis of keratinocytes could be another mechanism for the development of acanthosis in seborrheic keratoses. We therefore stained 65 seborrheic keratoses and 19 normal skin samples for the expression of anti-apoptotic bcl-2 protein (Figure 2c). Bcl-2 was predominantly expressed in the basal layers of the epidermis both in normal epidermis and seborrheic keratoses as reported previously.⁹ However, some control and seborrheic keratoses samples additionally revealed bcl-2 expression in suprabasal layers of the epidermis. The bcl-2 expression was scored from 0 (negative) to 3 (strong positive). The mean bcl-2 expression score was 1.6 ± 0.7 in normal epidermis, and 1.5 ± 0.7 in wild-type seborrheic keratoses. By contrast, seborrheic keratoses with *FGFR3* mutations revealed a mean bcl-2 expression score of 2.0 ± 0.8 . The difference of bcl-2 expression between wild type and mutant seborrheic keratoses was significant ($P = 0.02$). These results suggest a possible trend to an increased expression of anti-apoptotic bcl-2 protein in *FGFR3* mutant seborrheic keratoses.

Expression of FGFR3 Protein in Seborrheic Keratoses

Since *FGFR3* protein overexpression has been reported in other tumors and the expression in human seborrheic keratoses has not been analyzed

yet, we also investigated *FGFR3* protein expression in human seborrheic keratoses and control samples by immunohistochemistry. *FGFR3* protein was preferentially expressed in the basal layer of the epidermis both in seborrheic keratoses and normal skin (Figure 2d), but some samples additionally showed suprabasal staining. The mean *FGFR3* expression score was 1.6 ± 0.7 both in normal epidermis and in *FGFR3* wild-type seborrheic keratoses, while the expression score of the *FGFR3* mutant seborrheic keratoses was 1.9 ± 0.7 ($P = 0.048$). Immunohistochemistry shows that *FGFR3* protein is regularly expressed in the basal epidermis of human seborrheic keratoses and may show a slightly increased expression in seborrheic keratoses with *FGFR3* mutations.

Discussion

Although seborrheic keratoses are one of the most common skin tumors in man, their pathogenesis is still poorly understood. A causative role of somatic *FGFR3* mutations in human seborrheic keratoses resulting in a constitutive ligand-independent receptor activation has been reported.⁵ Epidermal nevi, which are benign congenital skin lesions and histologically very similar to seborrheic keratoses, show also *FGFR3* mutations as an underlying genetic basis.⁸ Therefore, evidence is growing that *FGFR3* mutations are important genetic alterations in the pathogenesis of benign acanthotic skin tumors.¹⁰

However, not all seborrheic keratoses and epidermal nevi display *FGFR3* mutations, suggesting the involvement of further genes. In the present study, we detected *FGFR3* mutations in 57% of seborrheic keratoses, which is within the reported range from 26 to 85%.^{5,6,11} Most of the *FGFR3* mutations in human skin tumors as well as in urothelial carcinoma and in the germline of patients with skeletal dysplasia syndromes appear to be restricted to several hot spots in exons 7, 10, and 15.^{5,6,8,12–14} Interestingly, all *FGFR3* mutations detected in human skin tumors so far are associated with thanatophoric dysplasia, SADDAN (severe achondroplasia with developmental delay and acanthosis nigricans) syndrome and Crouzon syndrome in germline. The *FGFR3* mutations causing the above-mentioned skeletal dysplasia syndromes are associated with a strong constitutive receptor activation.¹⁵ Compatible with these observations, patients with thanatophoric dysplasia, SADDAN syndrome and Crouzon syndrome develop acanthosis nigricans of the skin, a condition closely resembling both seborrheic keratoses and epidermal nevi in histopathology. By contrast, the G382R mutation causing achondroplasia in germline and probably representing the nucleotide with the highest mutation frequency in the human genome,¹³ has not been found in seborrheic keratoses and only

rarely in urothelial tumors. This mutation is linked with a less pronounced receptor activation,¹⁵ and achondroplasia patients usually do not show acanthosis nigricans of the skin. We have therefore hypothesized that the development of benign acanthotic skin tumors obviously requires a strong degree of FGFR3 activation.¹⁰ The data of the presented study further confirm this concept because the G382R mutation was not detected among the 65 seborrheic keratoses.

While an involvement of *FGFR3* mutations in the pathogenesis of human seborrheic keratoses has been shown, the time point for the occurrence of these mutations is unknown. Therefore, we analyzed whether *FGFR3* mutations are already present in flat (initial) seborrheic keratoses and therefore occur early, or are associated with an increased thickness of the tumors and represent therefore late genetic alterations mediating an increased vertical growth of flat seborrheic keratoses. Our results show that *FGFR3* mutations are already present in flat seborrheic keratoses. This finding indicates that the mutations may be early genetic events in the pathogenesis of at least a subset of seborrheic keratoses.

Interestingly, we found that the frequency of *FGFR3* mutations was significantly associated with an increased age. It has to be noted that the age in this study is defined as the time point of seborrheic keratoses removal and does not necessarily reflect the age of the first appearance of the lesions. However, individuals with *FGFR3* mutant seborrheic keratoses were, on average, 10 years older than individuals with wild-type seborrheic keratoses. This result suggests that age is a major risk factor for the occurrence of somatic *FGFR3* mutations in skin. One could speculate that cumulative exposure to environmental factors such as chronic UV-light exposure may contribute to *FGFR3* mutations in aging skin.

This hypothesis is further supported by the fact that in our study seborrheic keratoses localized on the head and neck revealed a significantly higher *FGFR3* mutation frequency than seborrheic keratoses localized on the trunk and extremities, correlating well with a higher cumulative lifetime UV-light exposure of the head and neck compared with the trunk. Furthermore, the R248C mutation was the most frequently detected mutation in our study, particularly in the head region. This mutation is a typical UV-signature mutation. It had been postulated that age and UV light are independent risk factors for the development of seborrheic keratoses.^{1,2} The results of this study suggest that these risk factors contribute not only to the pathogenesis of seborrheic keratoses in general, but particularly to the development of seborrheic keratoses caused by *FGFR3* mutations. However, the fact that in some families seborrheic keratoses appear to be autosomal dominant inherited suggests that the genetic background of an individual may be also an important factor for seborrheic keratoses development.^{16,17}

The details of FGFR3 signaling in wild-type and mutant epidermal cells remain elusive. It is supposed that FGFR3 signaling is cell type-specific.¹⁸ Activating *FGFR3* mutations in human skin may provide proliferative signals for the keratinocytes via the Ras-MAPK pathway. Moreover, anti-apoptotic pathways induced by recruitment of PI-3 kinase may result in a prolonged survival of the keratinocytes in *FGFR3* mutant seborrheic keratoses.^{10,19} Our immunohistochemical data show that seborrheic keratoses independent of their *FGFR3* status reveal a significantly increased cell proliferation compared with normal skin. A previous study reported also an increased Ki-67 expression in seborrheic keratoses compared to normal skin.²⁰ Expression of anti-apoptotic bcl-2 was significantly associated with *FGFR3* mutations in acanthotic seborrheic keratoses, suggesting that the anti-apoptotic PI-3K/Akt-pathway upstream of bcl-2 may be involved in addition to an increased proliferation. Another study found a significantly increased bcl-2 expression in human seborrheic keratoses compared with inflamed seborrheic keratoses and inverted follicular keratoses, but these authors did not investigate the *FGFR3* status of the lesions.²¹ We found that *FGFR3* mutations were associated with a slightly increased FGFR3 protein expression. This correlation was barely significant, and further studies have to confirm a potential FGFR3 protein overexpression in mutant seborrheic keratoses. FGFR3 mRNA and protein overexpression is known from urothelial carcinoma, and monoclonal antibodies against FGFR3 inhibited the proliferation of an urothelial cancer cell line.²² Likewise, FGFR3 protein overexpression in a thyroid cancer cell line led to the loss of cell density-dependent growth inhibition.²³

While alternative or additional genetic alterations in human seborrheic keratoses as well as details of the involved signaling pathways need to be further determined, the mutant FGFR3 receptor represents a promising pharmaceutical target for the topical treatment of multiple seborrheic keratoses, which are still a major disfiguring problem of aging skin.

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Conflict of interest

The authors declare no conflict of interest.

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