Activating mutations of the tyrosine kinase receptor FGFR3 are associated with benign skin tumors in mice and humans

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Specific germline activating point mutations in the gene encoding the tyrosine kinase receptor FGFR3 (fibroblast growth factor receptor 3) result in autosomal dominant human skeletal dysplasias. The identification in multiple myeloma and in two epithelial cancers—bladder and cervical carcinomas—of somatic *FGFR3* mutations identical to the germinal activating mutations found in skeletal dysplasias, together with functional studies, have suggested an oncogenic role for this receptor. Although acanthosis nigricans, a benign skin tumor, has been found in some syndromes associated with germinal activating mutations of *FGFR3*, the role of activated FGFR3 in the epidermis has never been investigated. Here, we targeted an activated receptor mutant (S249C FGFR3) to the basal cells of the epidermis of transgenic mice. Mice expressing the transgene developed benign epidermal tumors with no sign of malignancy. These skin lesions had features in common with acanthosis nigricans and other benign human skin tumors, including seborrheic keratosis, one of the most common benign epidermal tumors in humans. We therefore screened a series of 62 cases of seborrheic keratosis for *FGFR3* mutations. A large proportion of these tumors (39%) harbored somatic activating *FGFR3* mutations, identical to those associated with skeletal dysplasia syndromes and bladder and cervical neoplasms. Our findings directly implicate FGFR3 activation as a major cause of benign epidermal tumors in humans.

INTRODUCTION

Fibroblast growth factor receptor 3 (FGFR3) belongs to a class of transmembrane tyrosine kinase receptors (FGFR1–4) involved in signal transduction regulating cell growth, differentiation, migration, wound healing and angiogenesis, depending on target cell type and developmental stage. These receptors are glycoproteins composed of two or three extracellular immunoglobulin (Ig)-like domains, a transmembrane domain and a split tyrosine kinase domain. Ligand binding induces receptor dimerization, leading to phosphorylation of the kinase domain (1,2). Specific activating point mutations affecting different domains of FGFR3 result in autosomal dominant human skeletal dysplasias: craniosynostoses (Crouzonodermoskeletal syndrome and Muenke craniosynostosis) and dwarfing chondrodysplasias of various degrees of severity (hypochondroplasia, achondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans—also known as 'SADDAN'—and thanatophoric dysplasia) (3,4). The degree of activation of FGFR3 is

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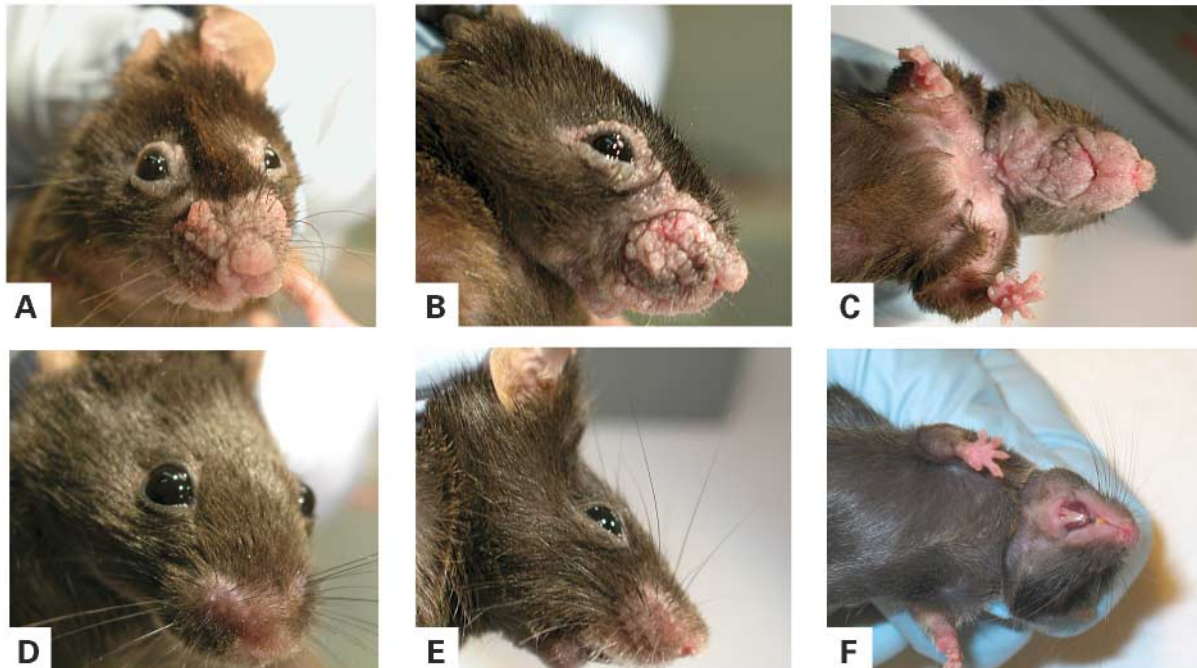
Figure 1. Phenotype of K5-(S249C)FGFR3 transgenic mice. (**A** and **B**) Two K5-(S249C)FGFR3 transgenic mice (line 79) at 6 months of age. These mice exhibit verrucous cauliflower-like lesions on the snout and the eyelids. These lesions are well demarcated, of symmetrical localization and occasionally display partial pigmentation, as shown in (B). (**C**) Throat and upper chest skin lesions on a 10-month-old K5-(S249C)FGFR3 transgenic mouse (line 79) are shown. The throat lesions resemble those on the snout in (A and B), areas of flat skin thickening with hair loss are clearly apparent on the upper chest. (**D**–**F**) Control littermates are shown below each transgenic mouse.

correlated with the severity of dwarfing chondrodysplasias (5). Functional studies have shown that some of these mutations activate the receptor either by generating a cysteine residue that can form an intermolecular disulfide bond leading to ligand-independent dimerization of the receptor (R248C) or by changing the conformation of the activation loop in the tyrosine kinase of the receptor (K650/652E) (3,4). Additional evidence consistent with FGFR3 playing a negative role in bone growth has been obtained in several mouse models including *Fgfr3*-deficient mice and mice harboring activating mutations in *FGFR3* (4). In contrast to this inhibitory role, an oncogenic role has been proposed for FGFR3 in cancer. Somatic activating *FGFR3* mutations identical to those found in skeletal dysplasias have been shown to be associated with multiple myeloma and more recently with epithelial malignancies (bladder and cervix carcinomas) (6–8). Functional studies have provided several lines of evidence supporting an oncogenic role for FGFR3: activated FGFR3 induces the malignant transformation of NIH3T3 fibroblasts (9,10) and generates lymphoid malignancies in mice (11). Although acanthosis nigricans, a benign skin epidermal tumor, has been found in some syndromes associated with germinal activating mutations of FGFR3 (Crouzonodermoskeletal syndrome, SADDAN syndrome and rare long-surviving thanatophoric dysplasia patients) (12–15), the role of activated FGFR3 in the epidermis has never been investigated.

We investigated the role of activated FGFR3 in the epidermis by targeting the expression of an activated receptor (S249C FGFR3) to the basal layer of the epidermis, using the bovine keratin 5 (K5) promoter (16,17). The S249C

mutation, which causes ligand-independent activation of the receptor, is the most common *FGFR3* mutation in carcinomas (8,18,19). This mutation also causes thanaphoric dysplasia, a syndrome associated with acanthosis nigricans in the rare long-term survivors (14,15).

RESULTS

Transgenic mice expressing mutated FGFR3 (FGFR3 S249C) in the epidermis develop benign skin tumors

We targeted the expression of a mutated form of FGFR3 (FGFR3 S249C) to the epidermis using the keratin 5 promoter. The K5-(S249C)FGFR3 construct was made by subcloning the FGFR3 S249C cDNA (see Materials and Methods) into a vector containing a 5.2 kb fragment of the bovine keratin 5 promoter, the rabbit β-globin intron 2 and the SV40 polyadenylation signal. This construct was then microinjected into fertilized B6D2F1 mouse oocytes. Among the seven founders obtained, four expressed the K5-(S249C)FGFR3 transgene in the skin (data not shown). Three of these four founders developed a skin phenotype. These three founders were viable and fertile and transmitted the transgene to their offspring in a Mendelian fashion. The offspring developed a phenotype indistinguishable from that of the founders. At the age of 3–4 months, the transgenic mice presented skin verrucous tumors on the eyelids and the snout (Fig. 1A and B), which then gradually extended from the snout to the throat. In animals aged >5 months, another type of lesion appeared on the upper trunk, characterized by a marked thickening of
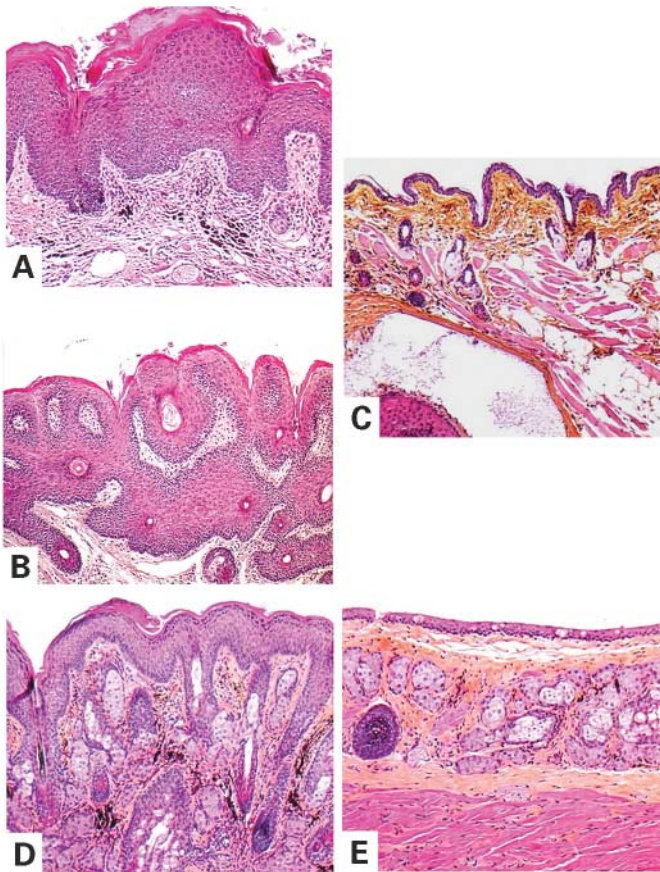
Figure 2. Tumor histology of K5-(S249C)FGFR3 transgenic mouse skin lesions. (**A**, **B** and **D**) Hematoxylin, eosin and saffron sections of skin lesions from the snout (A and B) and the trunk (D) of a transgenic K5-(S249C)FGFR3 mouse. (**C** and **E**) Sections of normal snout (C) and trunk (E) from a control littermate are shown for comparison. These skin lesions showed various degrees of acanthosis, papillomatosis and hyperkeratosis. Lesion in (B) is thicker than in (A) and shows interlacing strands (trabeculated aspect) of basaloid and squamous cells enclosing hair follicles. The parakeratosis and spongiosis observed in (A) were probably caused by itching and repetitive scratching of the lesion. Magnification 100 × .

Figure 3. K5-(S249C)FGFR3 transgenic mice, tumor cell proliferation, as assessed by BrdU labeling. Mice were injected with 5-bromodeoxyuridine (BrdU), and tissue sections were analyzed by immunohistochemical staining with an antibody against BrdU. Labeled cells were restricted to the basal layer in both transgenic K5-(S249C)FGFR3 mice (**A**) and wild-type littermates (**B**). Magnification 200 × .

the skin and hair loss (Fig. 1C). None of the skin lesions arising in the various transgenic lines showed any signs of regression. In some old mice, a thickening of the tongue and palate was observed. No lesions were observed in other tissues, although some expressed the transgene including the forestomach and the trachea (Supplementary Material Fig. S1).

Histological examination of the skin lesions revealed common features, irrespective of body site and gross appearance: (i) pronounced acanthosis (thickening of the nucleated layers of the epithelium), (ii) hyperkeratosis (thickening of the stratum corneum) with occasional parakeratosis (hyperkeratosis, with retention of nuclei) and (iii) papillomatosis (numerous projections of the dermal papillae) (Fig. 2). The most exophytic lesions of the snout displayed a trabeculated pattern, with rare keratin-filled invaginations and small keratin cysts, which generally surrounded a hair shaft (Fig. 2B). No abnormalities of the hair follicles or sebaceous glands were observed. No histological features of malignancy were detected: mitotic figures were rare and always localized
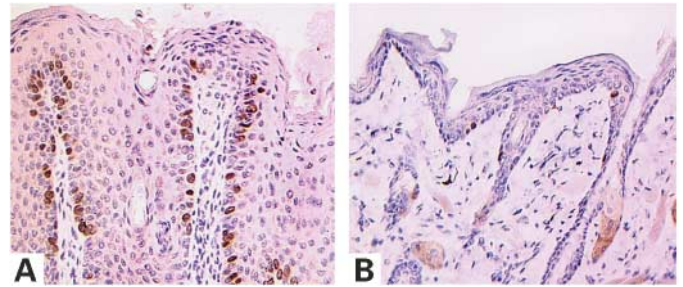
in the basal layer of the epidermis and cytological atypia was not observed. The absence of malignancy was evident, even in the lesions of transgenic mice >18 months old. Cell proliferation was studied by incorporation of the thymidine analog 5-bromo-2-deoxyuridine (BrdU). Consistent with the benign nature of the lesions, proliferating cells were restricted to the basal cell layer of the epidermis and to hair follicles in both transgenic and control mice (Fig. 3). However, the proportion of basal cells undergoing proliferation was significantly higher in transgenic mouse epidermis than in control skin (27 versus 1.8%, $P < 0.0001$).

Frequent *FGFR3* mutations are found in a common benign skin tumor in humans

The skin lesions observed in transgenic mice expressing mutated *FGFR3* displayed several histological features (acanthosis, hyperkeratosis and papillomatosis) common to benign human epidermal tumors including seborrheic keratosis, one of the most common benign epidermal tumors in humans. This lesion, which has no malignant potential, is mainly located on the trunk and face and presents as two histological types—the hyperkeratotic and the acanthotic types. Seborrheic keratoses tend to appear in middle age and become more common and numerous with advancing age.

We screened a series of 62 seborrheic keratoses for *FGFR3* mutations by using single strand conformation polymorphism (SSCP) analysis followed by DNA sequencing. The three regions of *FGFR3* (located in exon 7, 10, 15) known to harbor most of the point mutations previously described in multiple myeloma, bladder and cervical carcinomas and skeletal dysplasias were analyzed. We identified point mutations in 24 samples (39%) (Fig. 4 and Table 1). Matched constitutional DNA, available in 10 skin tumor cases with *FGFR3* mutations, contained wild-type sequences demonstrating the somatic nature of these mutations (Fig. 4) (data not shown).

We found no correlation among the presence of *FGFR3* mutations, the sex or age of the patients, the location of the tumors ($P = 0.21$) or the histological type (acanthotic or hyperkeratotic) ($P = 0.29$). Among the four patients for whom two seborrheic keratoses were analyzed, we found a *FGFR3* mutation in one of the two lesions for two patients
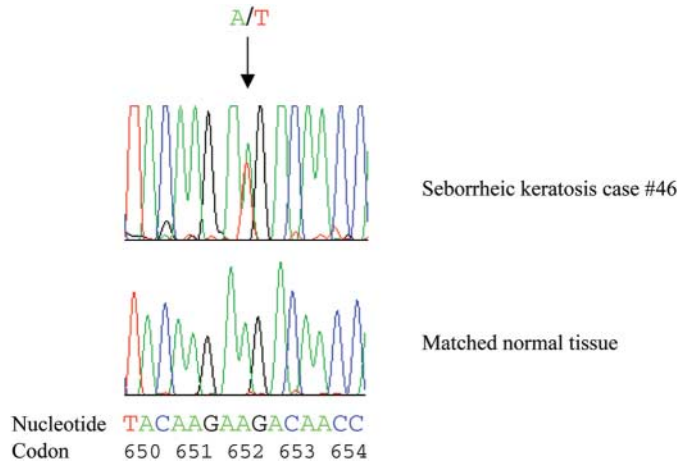
Figure 4. Example of a somatic *FGFR3* mutation in a seborrheic keratosis sample. Sequencing revealed a missense mutation in codon 652 (AAG–>ATG) of *FGFR3* in DNA from seborrheic keratosis 46 and a wild-type sequence in a normal matched DNA sample.

(cases 47a and b and 53a and b), no *FGFR3* mutation in either lesion for one patient (case 55a and b) and a different *FGFR3* mutation in each seborrheic keratosis for one patient (case 11a and b) (Table 1).

The 24 mutations identified affected seven different codons: R248C, S249C, G372C, S373C, Y375C, K652E and K652M (Table 2). All these mutations were identical to the constitutional mutations present in patients with thanatophoric dysplasia and SADDAN (3,20,21). With the exception of the S373C mutation, all the mutations have already been described as somatic mutations in multiple myeloma and in bladder and cervical carcinomas (6–8,18,19). The mutations in exon 7 (R248C and S249C) were located at the junction between extracellular Ig-like domains II and III of FGFR3, the mutations in exon 10 (G372C, S373C and Y375C) were located in the transmembrane domain and the mutations in exon 15 (K652E and K652M) were located in the second part of the intracellular tyrosine kinase domain of FGFR3.

DISCUSSION

Since the identification of mutations of *FGFR3* in human skeletal dysplasias, the role of FGFR3 in skeletal development has attracted considerable attention (4). The recent identification of somatic activating *FGFR3* mutations in multiple myeloma and in two epithelial cancers—bladder and cervix carcinomas—has raised the question of an oncogenic role of mutated *FGFR3* in tumorigenesis (6–8). In hematopoietic cells, functional studies have shown that mutated FGFR3 may contribute to the malignant phenotype (9–11). The oncogenic potential of mutated FGFR3 has yet to be investigated in bladder and cervix epithelia.

We show here that mutated FGFR3 can induce benign tumors with no malignant potential in mouse epidermis. This led us to identify frequent *FGFR3* mutations in seborrheic keratosis, one of the most frequent benign epidermal tumors in older individuals. Here, we screened part of the coding sequence of FGFR3 (exons 7, 10 and 15) containing most of

the mutations identified in severe forms of dwarfism and all the mutations already identified in bladder and cervix carcinomas. However, we cannot totally exclude the presence in seborrheic keratoses of *FGFR3* mutations outside these exons.

Although the clonal nature of seborrheic keratoses has been suggested (22), no molecular alterations had been reported in these lesions. We report for the first time a molecular alteration associated with this benign skin tumor. The *FGFR3* mutations that we found in seborrheic keratosis are the same as those found in thanatophoric dysplasia and SADDAN syndrome. Interestingly, the rare long-surviving thanatophoric dysplasia patients and all the patients with SADDAN syndrome develop acanthosis nigricans, a benign skin tumor sharing histological similarities with seborrheic keratosis and with the mouse skin lesions observed in our transgenic mice. Our results in mice strongly suggest that the occurrence of acanthosis nigricans observed in these human skeletal disorders results directly from the expression of mutated *FGFR3* in the epidermis.

We expressed an activated form of FGFR3 in the mouse epidermis by targeting the FGFR3 S249C transgene to the epidermis with the keratin 5 promoter. A different approach—targeted homologous recombination—has been used in previous studies to generate several mouse models harboring a mutated endogenous *FGFR3* gene (23–26). The various mutations inserted corresponded to mutations found in skeletal dysplasias, carcinomas and, as shown here, seborrheic keratoses. Surprisingly, although the endogenous mouse gene is expressed in the skin, no skin lesions have been reported in these models. The absence of a skin phenotype may be due to the genetic background of the mice used in these models, the level of expression of the mouse *FGFR3* gene, differences in the regulation of the mouse and human promoters or slight differences between the mouse and the human FGFR3.

The skin lesions observed in our transgenic mouse model have several histological features in common with seborrheic keratoses (acanthosis, hyperkeratosis and papillomatosis). Human seborrheic keratoses characteristically display keratin-filled structures (horn cysts and pseudo-horn cysts). In the mouse model, horn cysts were present in some thick lesions, but were less numerous and smaller than those in human seborrheic keratoses. Furthermore, in mice, these cysts always surrounded a hair. The differences between human and mouse lesions may simply correspond to a difference between species. They may also be due to mutated FGFR3 being under the control of the keratin 5 promoter in the mouse model, whereas mutated FGFR3 is under the control of its own promoter in human seborrheic keratoses. The FGFR3 promoter gives suprabasal expression in resting human skin and basal expression after wounding (27). In human seborrheic keratosis, FGFR3 expression was observed in both the basal and the suprabasal layers. In transgenic mouse lesions, the mutated FGFR3 was observed in the basal layer and in the first suprabasal layers (Claire Dunois-Lardé and Jean-Marie Gasc, unpublished results).

The induction of benign skin tumors by *FGFR3* mutations in mice, the occurrence of acanthosis nigricans in skeletal dysplasia syndromes caused by germinal *FGFR3* mutations and the identification of somatic *FGFR3* mutations in seborrheic

Table 1. Clinical data and FGFR3 mutations found in the 62 seborrheic keratoses

Case #	Age (yr)	Sex	Location of the tumor	Size (mm)	Histological type	FGFR3 codon	*FGFR3* Mutation
1	73	F	Trunk	10	Acanthotic		wt
2	64	F	Face	10	Acanthotic	248	CGC → TGC
3	76	F	Extremity	9	Hyperkeratotic		wt
4	51	F	Extremity	8	Hyperkeratotic		wt
5	47	M	Face	12	Acanthotic		wt
6	51	M	Face	8	Acanthotic	373	AGT → TGT
7	46	F	Extremity	8	Acanthotic		wt
8	56	M	Trunk	10	Acanthotic		wt
9	54	M	Face	9	Hyperkeratotic	248	CGC → TGC
10	76	F	Trunk	9	Acanthotic	249	TCC → TGC
11a	91	F	Trunk	12	Acanthotic	249	TCC → TGC
b			Trunk	15	Acanthotic	375	TAT → TGT
12	59	F	Trunk	15	Acanthotic		wt
13	86	M	Face	6	Hyperkeratotic		wt
14	71	M	Face	8	Acanthotic		wt
15	66	F	Trunk	8	Hyperkeratotic		wt
16	54	F	Face	10	Acanthotic	372	GGC → TGC
17	53	F	Trunk	7	Acanthotic		wt
18	84	F	Trunk	8	Hyperkeratotic	248	CGC → TGC
19	43	F	Face	12	Acanthotic	652	AAG → ATG
20	59	F	Trunk	15	Acanthotic		wt
21	67	F	Face	6	Acanthotic	372	GGC → TGC
22	57	F	Trunk	6	Hyperkeratotic		wt
23	63	F	Trunk	10	Acanthotic		wt
24	44	F	Trunk	4	Hyperkeratotic		wt
25	72	F	Trunk	13	Acanthotic	375	TAT → TGT
26	74	M	Face	6	Acanthotic		wt
27	40	F	Trunk	6	Acanthotic		wt
28	88	F	Trunk	7	Hyperkeratotic		wt
29	67	F	Face	7	Acanthotic		wt
30	66	F	Trunk	8	Hyperkeratotic		wt
31	85	M	Trunk	6	Acanthotic	248	CGC → TGC
32	83	F	Trunk	5	Hyperkeratotic		wt
33	67	F	Face	8	Acanthotic	249	TCC → TGC
34	31	F	Trunk	3	Acanthotic	248	CGC → TGC
35	76	F	Face	10	Hyperkeratotic		wt
36	79	F	Face	4	Acanthotic		wt
37	49	F	Trunk	6	Hyperkeratotic		wt
38	63	F	Face	15	Hyperkeratotic		wt
39	76	F	Trunk	6	Acanthotic		wt
40	52	F	Face	14	Hyperkeratotic		wt
41	56	F	Trunk	10	Acanthotic		wt
42	59	M	Face	18	Acanthotic		wt
43	83	F	Trunk	6	Hyperkeratotic	372	GGC → TGC
44	30	F	Trunk	10	Hyperkeratotic		wt
45	77	F	Trunk	4	Hyperkeratotic		wt
46	31	F	Trunk	5	Acanthotic	652	AAG → ATG
47a	79	M	Face	11	Hyperkeratotic	373	AGT → TGT
b			Face	4	Hyperkeratotic		wt
48	67	F	Trunk	6	Acanthotic	375	TAT → TGT
49	65	F	Trunk	15	Hyperkeratotic	373	AGT → TGT
50	79	F	Trunk	7	Hyperkeratotic	652	AAG → ATG
51	75	F	Face	14	Hyperkeratotic		wt
52	70	F	Face	15	Acanthotic	249	TCC → TGC
53a	79	F	Face	6	Hyperkeratotic	652	AAG → GAG
b			Face	11	Hyperkeratotic		wt
54	45	F	Trunk	12	Acanthotic		wt
55a	46	M	Extremity	9	Hyperkeratotic		wt
b			Trunk	6	Acanthotic		wt
56	76	F	Face	9	Acanthotic		wt
57	31	M	Face	4	Acanthotic	375	TAT → TGT
58	75	M	Face	10	Hyperkeratotic	249	TCC → TGC

F: female; M: male; wt: wild-type.
Two seborrheic keratoses were analyzed for cases 11, 47, 53 and 55.

Table 2. FGFR3 mutations in 62 seborrheic keratoses

Codon	nt Position	Exon	Mutation	Predicted effect	Number of tumors
248	742	7	CGC → TGC	Arg → Cys	5
249	746	7	TCC → TGC	Ser → Cys	5
372 (370)	1114	10	GGC → TGC	Gly → Cys	3
373 (371)	1117	10	AGT → TGT	Ser → Cys	3
375 (373)	1124	10	TAT → TGT	Tyr → Cys	4
652 (650)	1954	15	AAG → GAG	Lys → Glu	1
652 (650)	1955	15	AAG → ATG	Lys → Met	3

Codons and mutated nucleotides (nt position) are numbered according to the open reading frame of the FGFR3b cDNA. This isoform, which is the predominant form in epithelial cells, contains two more amino acids than the FGFR3c isoform, which is found in bone. Codon numbers according to the open reading frame of the FGFR3c cDNA are indicated in brackets.

keratoses support a role for mutated FGFR3 in benign epidermal tumorigenesis. The fact that we did not identify *FGFR3* mutations in a series of epidermal carcinomas (23 squamous cell carcinomas and 22 basal cell carcinomas) (28) (data not shown) suggests that the role of activated FGFR3 in the epidermis is restricted to benign tumorigenesis. In the bladder, although *FGFR3* mutations are found in a significant proportion of muscle-invasive carcinomas (15% of cases), it should be noted that such mutations occur at high frequency (>70% of cases) in low stage, low grade bladder carcinomas (TaG1–TaG2) (18,29). In addition, a high frequency of *FGFR3* mutations was recently detected in urothelial papilloma, a rare entity with little or no risk of developing urothelial carcinomas (30,31).

The transgenic mouse model we have set up should be useful for studying the mechanisms of benign tumorigenesis and for developing new therapies targeting mutated FGFR3. Although not life threatening, seborrheic keratoses can be troublesome and annoying to the patient. They may become irritated and itch. Often unattractive, or even disfiguring, these lesions have a negative psychological impact—daily reminders of aging. Anti-tyrosine kinase drugs have recently been used successfully to treat several malignancies (32). The identification of activating mutations of a tyrosine kinase receptor in a significant proportion of seborrheic keratoses may provide new opportunities for the treatment of this condition.

MATERIALS AND METHODS

Generation of K5-(S249C)FGFR3 transgenic mice

We targeted the expression of an activated FGFR3 carrying the S249C mutation to the epidermis in mice, using the 5′-regulatory region of the bovine keratin 5 gene (16). The FGFR3b splice variant was used, as this is the main form of FGFR3 in the murine epidermis and human epithelia (33) (unpublished data). The S249C mutation, which causes ligand-independent activation of the receptor, is the most common mutation in carcinomas (8). The coding sequence of the human FGFR3b cDNA was amplified by RT–PCR from RNA of a normal urothelium, using the primers

5′-CGGGGCTGCCTGAGGAC-3′ and 5′-TGCTAGGGACC CCTCACATT-3′. The PCR product was inserted into a cytomegalovirus promoter-driven pcDNAI/Neo expression vector (Invitrogen, San Diego, CA, USA). The S249C mutation was then introduced into this cloned FGFR3b cDNA as follows: a 729 bp region of the FGFR3b cDNA carrying the S249C mutation was amplified by RT–PCR from RNA of a bladder carcinoma, using the primers 5′-CGTCGTGGAGAACAAG TTTGGCAG-3′ and 5′-CCGAGACAGCTCCCATTTG-3′. The *Xho*1–*Aor*51H1 fragment containing the mutation was excised from the PCR product and inserted in place of the corresponding sequence in the non-mutated FGFR3b construct. The pK5-(S249C)FGFR3b construct was then obtained by inserting the mutated FGFR3b cDNA excised with *Xba*I and *Hin*dIII into the *Sna*BI site of the pBK5 expression vector containing the 5.2 kb bovine keratin 5 (K5) regulatory sequences, β-globin intron 2 and the 3′ polyadenylation sequences of SV40. All PCR-generated segments were verified by sequencing both strands. The K5-(S249C)FGFR3 construct excised with *Sal*I and *Not*I was purified and micro-injected into fertilized B6D2F1 oocytes. Genomic DNA was extracted from mouse tails and screened by PCR for integration of the transgene. The care, housing and handling of the mice conformed to the recommendations of the French Ethics Committee and were overseen by authorized investigators.

Histology of mouse tissues

Tissue samples from transgenic mice and wild-type littermates matched with respect to sex and body site were collected and fixed by overnight incubation at room temperature in freshly prepared Methacarn (6 vol absolute methanol/3 vol chloroform/1 vol acetic acid). The samples were embedded in paraffin, and sections (5 μm) were cut and stained with hematoxylin–eosin–saffron for histological analysis.

BrdU labeling

Transgenic mice ($n = 5$) from line 79 and control littermates ($n = 5$) were injected (i.p.), with 0.25 mg g^{-1} body weight BrdU (Sigma). They were sacrificed 2 h later. BrdU immunohistochemistry was carried out with the BrdU *in situ* detection kit (BD PharMingen, San Diego, CA, USA), using the reagents and conditions suggested by the manufacturer. The number of BrdU-labeled cells among 300 cells from the basal epidermis layer was determined for each mouse.

Human skin samples

Alcohol/formalin/acetic acid-fixed paraffin-embedded specimens of seborrheic keratoses were obtained from the Pathology Department of the Institut Curie and cut into 8 μm sections. One section was used for hematoxylin–eosin–saffron staining, for distinguishing the tumor cell areas and their subsequent microdissection from the remaining unstained sections. Genomic DNA was isolated using the QIAmp tissue kit (Qiagen), according to the manufacturer's instructions.

Mutation analyses

We used SSCP and sequencing to analyze the three regions of *FGFR3* in exons 7, 10 and 15 known to harbor most of the point mutations previously described in multiple myeloma, bladder and cervix carcinomas and skeletal dysplasias. SSCP and sequence analysis were performed as previously described (18), except that the following primer sequences were used: 5′-AGTGGCGGTGGTGGTGAGGGAG-3′ and 5′-GACGTT CCACATGTCACTGCGTGT-3′ for exon 7; 5′-CCTCAACG CCCATGTCTTTGCAGC-3′ and 5′-GTTCTAGAGGGCGAA GGGCGAGTTC-3′ for exon 10 and 5′-TGGTGACCGAGGA CAACGTGATG-3′ and 5′-GTTGTGGCGGAAGGGTGTGG GA-3′ for exon 15.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

ACKNOWLEDGEMENTS

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