A Mutation in the Serum and Glucocorticoid-Inducible Kinase-Like Kinase (Sgkl) Gene is Associated with Defective Hair Growth in Mice

Kentaro Masujin,1 Taro Okada,2 Takehito Tsuji,1 Yoshiyuki Ishii,2 Kaoru Takano,3 Junichiro Matsuda,3 Atsuo Ogura,4 and Tetsuo Kunieda1,*

Graduate School of Natural Science and Technology, Okayama University, Tsushima-naka, Okayama, 700-8530, Japan,1 Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0857, Japan,2 National Institute of Infectious Diseases, Shinjuku-ku, Tokyo 162-8640, Japan,3 and RIKEN Bioresource Center, Tsukuba, Ibaraki 305-0074, Japan4

(Received 5 August 2004; revised 7 October 2004)

Abstract

YPC is a mutant mouse strain with defective hair growth characterized by thin, short hairs and poorly developed hair bulbs and dermal papillae. To identify the gene associated with the phenotype, we performed genome-wide linkage analysis using 1010 backcross progeny and 123 microsatellite markers covering all chromosomes. The mutant locus (ypc) was mapped to a 0.2-cM region in the proximal part of mouse chromosome 1. This 0.2-cM region corresponds to a 450-kb region of genome sequence that contains two genes with known functions and five ESTs or predicted genes with unknown functions. Sequence analysis revealed a single C-to-A nucleotide substitution at nucleotide 1382 in the Sgkl gene, causing a nonsense mutation at codon 461. Sgkl encodes serum and glucocorticoid-inducible kinase-like kinase (SGKL), which belongs to a subfamily of serine/threonine protein kinases and has been suggested to have a role downstream of lipid signals produced by activation of phosphoinositide 3-kinase (PI3K). In the mutant SGKL, a serine residue in the C-terminal end of the protein (Ser486), which is indispensable for activation of SGKL upon phosphorylation, is abolished by premature termination. Specific expression of the Sgkl gene in the inner root sheath of growing hair follicles was also identified by in situ hybridization. Therefore, we concluded that the nucleotide substitution in the Sgkl gene is the causative mutation for defective hair growth in the ypc mutant mouse and that the signaling pathway involving SGKL plays an essential role in mammalian hair development.

Key words: Hair follicle; SGKL/SGK3/CISK; WNT signaling; Mutant mouse; IRS

1. Introduction

Hair follicle morphogenesis and the hair growth cycle are complex processes dependent on a series of mesenchymal-epithelial interactions in skin.1 Reciprocal exchange of signals between dermal and epidermal cells of skin regulates the formation of hair placodes during embryonic development, and it also regulates cyclic transformation of the growth (anagen), regression (catagen), and quiescent (telogen) phases in the hair cycle in adult skin. As these processes show a high degree of organization and self-renewal, hair follicle development and hair cycling are thought to be excellent models for investigating the molecular mechanisms of mesenchymal-epithelial interactions.

Numerous growth factors and cytokines have been shown to be involved in morphogenesis and cycling of hair follicles. WNT,2,3 TGFα,4,5 BMPs,6,7 and FGFs8 in particular, as well as their signal transduction molecules,9,10 play essential roles in these processes. Experiments with transgenic mice or those with knockout mutations in these genes have demonstrated a number of abnormalities in morphogenesis and cycling of hair follicles, including a short-hair phenotype and cyclical balding in transgenic mice overexpressing the Wnt3 gene in skin3 and abnormally long hair in Fgf5 knockout mice, which is caused by defective regulation of the hair cycle.8 On the other hand, spontaneous mutant mouse strains showing abnormalities in hair morphogenesis have also provided useful information on the molecular mechanisms of these processes. For example, the hairless (hr) mutant, which
shows complete loss of hair and degeneration of hair follicles in the first regression phase, is caused by a retroviral insertion in the gene encoding a putative zinc-finger transcription factor,\textsuperscript{11} and the waved-2 (\textit{wa-2}) mouse phenotype, characterized by curly whiskers, waved hairs, and open eyelids at birth, is due to a single-base mutation in the \textit{EGF}/\textit{TGF\alpha} receptor gene (\textit{Egfr}).\textsuperscript{12}

YPC is a mutant mouse strain established from a Swiss albino mouse colony at the National Institute of Health, Japan, which shows defective hair growth controlled by an autosomal single recessive gene (\textit{ypc}). Homozygous \textit{ypc}/\textit{ypc} mice show thin and short hairs, wavy vibrissae (Fig. 1), and poorly developed hair bulbs and dermal papillae, but structures of the epidermis, dermis, and the sebaceous glands are normal.\textsuperscript{13} These phenotypes of \textit{ypc}/\textit{ypc} mice are unique and distinct from other mutant mice with hair abnormalities. Therefore, the YPC strain could be an excellent model for investigating the molecular mechanisms underlying the morphogenesis and cycling of hair follicles and the mesenchymal-epithelial interaction.

In the present study, we mapped the \textit{ypc} locus to the proximal region of mouse chromosome 1 by linkage analysis, sequenced genes in the critical region, and finally identified the causative mutation in a gene associated with intracellular transduction of signals implicated in the morphogenesis and cycling of hair follicles.

2. Materials and Methods

2.1. Animals

In the present study, we used mice of YPC and JF1/Msf strains maintained at Okayama University and those of an ICR colony. The YPC strain was provided by the National Institute for Infectious Diseases. The JF1/Msf strain, used as mating partners in the linkage analysis, was provided by the National Institute of Genetics. As the JF1/Msf strain is derived from \textit{Mus musculus molossinus} and has distance from most of the inbred laboratory strains, this strain is useful for linkage analysis. The mice of the ICR colony, which were used as normal controls for sequence and histological analyses, were purchased from Japan CLEA Inc.

2.2. Histological analysis

Mid-dorsal skin samples of the mutant and normal mice at postnatal day 0 (P0), P3, and P5 were fixed in 10\% neutral-buffered formalin, dehydrated through an alcohol gradient, and embedded in paraffin. Longitudinal sections of hair follicles 4 \textmu m thick were stained with hematoxylin and eosin (HE). For high-resolution histology, small pieces of the skin were fixed in 2.5\% glutaraldehyde and 2\% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB), postfixed in 1\% osmium tetroxide, embedded in Epon, and sectioned at a thickness of 1 \textmu m. These sections were stained with toluidine blue.

2.3. Experimental cross and linkage analysis

A total of 1010 backcross progeny, including 496 affected and 514 unaffected mice obtained by crossing the YPC and JF1/Msf strains, were used for linkage analysis. Homozygous \textit{ypc}/\textit{ypc} mice of the YPC strain were mated with +/+ JF1/Msf mice and the resultant F\textsubscript{1} (\textit{ypc}/+) mice were mated with homozygous \textit{ypc}/\textit{ypc} mice of the YPC strain to obtain the 1010 backcross progeny. Genomic DNA samples of the backcross progeny were obtained from livers by phenol/chloroform extraction and the genotypes of 123 microsatellite markers on mouse chromosomes were determined. Aliquots of 20 ng of genomic DNA were subjected to PCR amplification in 10-\mu reaction mixtures, containing 1.5 mM Mg\textsuperscript{2+}, with 40 cycles consisting of 94\degreeC for 30 sec, 55\degreeC for 30 sec, and 72\degreeC for 60 sec. The PCR products were typed by polyacrylamide or agarose gel electrophoresis. The nucleotide sequences of the primers for these microsatellite markers were obtained from the Mouse Genome Informatics sequence database (http://www.informatics.jax.org/). The recombination fractions were calculated and the order of loci was determined using MapManager QT Ver. 3.0.

2.4. New microsatellite markers on the critical region

New microsatellite markers were obtained by searching GA/TC repeats on the sequence data of the region of mouse chromosome 1 close to the \textit{ypc} locus. The sequence data were obtained from the mouse genome sequence in the NCBI database (http://www.ncbi.nlm.nih.gov). Among the GA/TC repeats found in this region, we chose the GA/TC repeats located inside or close to functional genes, and a total of 14 new microsatellite markers...
(D1Mok1 to D1Mok14) were obtained. The nucleotide sequences of the primers for these markers are shown in Table 1. The genotypes of these microsatellite markers in the backcross progeny were determined with the same conditions as described above.

2.5. Cloning and sequence analysis

The entire coding regions of two genes and an EST were amplified from skin RNA of normal and mutant mice by RT-PCR. Total RNA samples were obtained by the acid guanidinium-phenol-chloroform (AGPC) method. First-strand cDNA was synthesized from 10 µg of total RNA, using oligo d(T) primer and Superscript II reverse transcriptase (Gibco-BRL, Gaithersburg, MD) and used for PCR amplification with primer pairs for these genes. The nucleotide sequences of these primers obtained from EMBL/GenBank databases are shown in Table 2. PCR amplification was carried out for 30 to 40 cycles, consisting of 94°C for 45 sec, 50–58°C for 45 sec, and 72°C for 120 sec in 10-µl reaction mixtures containing 1.5 mM Mg²⁺. The amplified fragments were cloned into the pGEM-T Easy Vector system (Promega, Madison, WI) and their nucleotide sequences were determined by the dideoxy chain termination method with a Hitachi SQ5500 automated DNA sequencer. To distinguish authentic base substitutions from PCR errors, at least five independent clones were completely sequenced in both orientations.

2.6. Genotyping of the Sgkl gene

To confirm the correlation between the nucleotide substitution and the phenotype, genomic DNAs obtained from mice of the YPC strain, the backcross progeny, and other inbred strains including JF1/Msf, C57BL/6J, BALB/c, and ICR were used for genotyping the Sgkl gene. The 380-bp region flanking the substitution was amplified by PCR using a pair of primers 5′-TGAAAGGAAGGTAAGGTGAA-3′ and 5′-GCCCTATTTCTTGCATACAG-3′. The PCR products were digested with MseI restriction endonuclease and the digests were electrophoresed through 2% agarose gel in TBE buffer and visualized with ethidium bromide staining.

2.7. In situ hybridization

For in situ hybridization, DIG-labeled sense and antisense Sgkl riboprobes were synthesized using T7 and SP6 RNA polymerases (Roche Diagnostics, Mannheim, Germany) from the vector pCRII-TOPO (Invitrogen, Carlsbad, CA) containing a 618-bp fragment of the mouse Sgkl gene (nt 311 to 928, NM_133220). In situ hybridization was carried out as described previously with some modifications. In brief, 4% PFA-fixed mid-dorsal skin paraffin sections 8-µm thick were acetylated, treated with 0.2 M HCl, digested with 10 µg/ml proteinase K for 20 min, and then fixed with 4% PFA. Following prehybridization, sections were hybridized with DIG-labeled sense or antisense probes at 57°C overnight, and then the sections were washed in 50% formamide/SSC, digested with 20 µg/ml RNase A, and re-washed in SSC.

Table 1. Microsatellite markers for the ypc critical region.

| Microsatellite markers | Primer sequences | Product size (bp) |
|------------------------|------------------|------------------|
| D1Mok1                 | ACTAATAAGAAGATGCAGCTGCC | 283             |
| D1Mok2                 | GTGTCGAAACAAATCTTCGAAGG | 252             |
| D1Mok3                 | TCTTGAATCTGGAAGATATGCC | 280             |
| D1Mok4                 | CCACTCTTGTAACCATCGAGG | 276             |
| D1Mok5                 | GACCAACATGACTACACCACCC | 256             |
| D1Mok6                 | AGTACAGAGACAGCTTGGTGAC | 254             |
| D1Mok7                 | TACGTCTTTGGCCAGGAAATACG | 241             |
| D1Mok8                 | CAAGGCGCAACCCAGCTTACAGG | 302             |
| D1Mok9                 | GGCAGTGACCTCTCCCTCCT | 355             |
| D1Mok10                | GATCCAGAACTCTGTAAGAGCC | 316             |
| D1Mok11                | ACAACACTTCTGACATTCAGTCC | 375             |
| D1Mok12                | GGAGTGGTAATCCCTATGACCC | 418             |
| D1Mok13                | TGGAACTACCCAGCTTATACCC | 216             |
| D1Mok14                | CGTCACCTGTGAGACTCTGACAGG | 361             |
A Mutation in Sgkl Gene Causes Defective Hair Growth

Table 2. Primers for genes in the ypc critical region.

| Genes   | Fragments | Forward 5'-3' | Reverse 5'-3' | Product size (bp) |
|---------|-----------|---------------|---------------|------------------|
| Sgkl    | Sgkl-1    | GAATTCAGGGCTTCACAGGA | GCCCACAGAGACCAGAATCT | 340              |
| Sgkl-2  | ACTACAAGGAGAGCTGGCCA | TGGAGTAAATGTGCTCGTCC | 410              |
| Sgkl-3  | GAGACACCACAGAGACCTAGT | AACCTGCTCTGGGTCTCAAGG | 438              |
| Sgkl-4  | CCTCAAGGGAAGAGGTCTTTCT | ACTCTGTGTCAGAATTTAAGG | 399              |
| Sgkl-5  | GCGAGATGTTGCTGAAAGGA | GGTTCGCTGAAATGGGAAAGTTG | 425              |
| Mybl1   | Mybl1-1   | CTCTGGTCTACCTGGAAAAG | GTCATCCGATCTCTCGTC | 260              |
| Mybl1-2 | CATGATTATGAGATCTCTCACC | GTCAGGAAGACTCTTCC | 374              |
| Mybl1-3 | GTGAGAGAAGAGATTGGCA | TACCCAGGATGCTGAACAGG | 327              |
| Mybl1-4 | TGAGCATTGCAACACCCAGG | GTGGGTGATATCTGAGGC | 367              |
| Mybl1-5 | TGGAGGAAACACACTTCAGG | ATAGCTTACTGCTCCACC | 349              |
| Mybl1-6 | GTACGCTTTGGTACATGGAAGG | GATTTGGGGGTGCTTCC | 367              |
| Mybl1-7 | CAACCAAAATTGGGGCAGG | CCACGCTCTGAAAGCAAGG | 368              |
| Mybl1-8 | CTCCTGCTATGGAAGAAGG | GGTTAGGCGATTTATCCAG | 443              |
| 1700011J18Rik | 18Rik-1 | GGAAGGACTCGGACACCAAGA | GGTTCGCTGAAAGGAGAGG | 307              |
| 18Rik-2 | CGGAGCCTCGTGACTGAC | GAGAGACCTGACTGAC | 322              |
| 18Rik-3 | GGGAGAGCAATACATTGGC | CATGAAATACCTTTACACTGC | 284              |

Following antigen blocking, the sections were incubated with alkaline phosphatase conjugated anti-DIG antibody (1:500, Roche Diagnostics) at 4°C overnight. Positive signals were visualized by BCIP (5-bromo-4-chloro-3-indolyl phosphate) and NBT (nitroblue tetrazolium).

3. Results

3.1. Histological examinations

As shown in Fig. 1, the homozygous ypc/ycop mice showed poorly developed hairs and wavy vibrissae. The hair shafts of ypc/ycop mice were thinner and shorter than those of normal mice and had a disorganized hair medulla. To investigate the precise defects in postnatal hair follicle development in the ypc/ycop mouse, we performed histological examinations of mid-dorsal skin of ypc/ycop and +/+ mice at P0, P3, and P5. While no histological differences were observed between ypc/ycop and +/+ mice at P0 (Figs. 2A and 2B), the hair follicles in the ypc/ycop mice at P3 showed irregular structures characterized by small hair bulbs, narrow and immature hair shafts, and lack of uniform orientation (Figs. 2C and 2D). These features were also observed at P5, and the length of hair follicles of ypc/ycop mice was shorter than that in +/+ mice at this stage (Figs. 2E and 2F). At high magnification, toluidine blue-stained sections showed a reduced number of matrix cells, disorganized hair medulla, increased thickness of outer root sheath (ORS), and decreased thickness of inner root sheath (IRS). In conclusion, histological examination of ypc/ycop mice revealed abnormal postnatal development of the hair follicles, including small hair

Figure 2. Histological findings of postnatal day 0 (P0), P3, and P5 ypc/ycop mice. At P0, no morphological differences were observed between ypc/ycop and +/+ mice (A, B). At P3, smaller hair bulbs, disorganized hair shafts, and a lack of uniform orientation were observed in ypc/ycop (C, D). At P5, follicles of ypc/ycop mice still showed the structural abnormalities, and the length of the hair follicles became shorter than that in ICR (E, F). Toluidine blue-stained sections of ypc/ycop mice with high magnification showed a reduced number of matrix cells, disorganized hair medulla, increased thickness of outer root sheath (ORS), and decreased thickness of inner root sheath (IRS). Outer and inner yellow lines denote the boundary between ORS and IRS, and IRS and hair shaft, respectively. Bars: 200 µm (A to F) and 50 µm (G and H).
3.2. Linkage analysis

To localize the ypc locus on mouse chromosomes, initial linkage analysis was performed using 45 backcross progeny, including 17 affected and 28 normal mice. By typing the 100 microsatellite markers covering the entire mouse genome, significant linkage was observed between the ypc locus and the microsatellite marker D1Mit4 on the proximal region of chromosome 1. No significant linkage was observed between the ypc locus and any of the other 99 markers examined. These findings indicated that ypc is localized in the proximal region of mouse chromosome 1. We next performed fine linkage mapping of the ypc locus using a total of 1010 backcross progeny and 23 microsatellite markers in this region, including 14 new microsatellite markers (D1Mok1 to D1Mok14) obtained by searching for GA/TC repeats in the sequence data of this region. The segregation of alleles of these markers in the F2 progeny is shown in Fig. 3A. Fine linkage mapping revealed that the ypc locus is positioned in a 0.2-cM region between microsatellite markers D1Mok5 and D1Mok8, and no recombination was observed between ypc and D1Mok6 or D1Mok7. As shown in Fig. 3B, comparison of the linkage map with the published sequence of the mouse genome showed that the 0.2-cM region corresponds to a 450-kb region containing two genes with known function (mybl1 and Sgkl), three ESTs (311003E14Rik, 5730538E15Rik, and 1700011J18Rik), and two sequences predicted to be coding sequences of genes (LOC329092 and 6030422M02).

3.3. Sequence analysis of the genes located in the critical region

We sequenced the entire coding regions of the mybl1 and Sgkl genes as well as 1700011J18Rik in ypc/ypc and +/+ mice by RT-PCR and cloning of the amplified fragments. As the features of the other two expressed sequence tags (ESTs) and two predicted genes were unlikely to be functional and these sequences are not conserved between mouse and human, they were not sequenced. Comparison of the nucleotide sequences of these genes and EST between ypc/ypc and +/+ mice revealed a single C-to-A nucleotide substitution in the Sgkl gene, which encodes serum and glucocorticoid-inducible kinase-like kinase (SGKL). The nucleotide substitution occurred at nucleotide 1382, and resulted in premature termination at codon 461 (Fig. 4). Therefore, the SGKL protein in ypc/ypc mutant mice lacks the 35 amino acid residues of the C-terminal end of the protein. The C-terminal end is highly conserved among mouse and human and contains a serine residue for phosphorylation, which is indispensable in activation of SGKL. No mutation in the nucleotide sequence affecting the amino acid sequence of the proteins, including missense, nonsense, and frame shift mutations, were observed in the remaining gene or EST. These findings clearly indicate that the nonsense mutation of the Sgkl gene is associated with the mutant mouse phenotype.

3.4. Association between the nucleotide substitution and the phenotype

To confirm the association between the nonsense mutation and the phenotype, the genotype of the Sgkl gene was examined in various mice of the YPC strain, the back cross progeny, and other inbred strains. A 380-bp region including the nucleotide substitution was amplified by PCR. Since the nucleotide substitution is located in a recognition sequence of Mse I endonuclease, the amplified fragment of the mutant allele has an Mse I cleavage site, while that of the wild-type allele has no Mse I cleavage site. Digestion of the amplified fragment with Mse I demonstrated that all mice of the YPC strain and all affected mice of the backcross progeny gave digested fragments, all phenotypically normal mice of the backcross progeny gave both the digested and undigested fragments, and all mice of the other strains gave the undigested fragment. Therefore, the nucleotide substitution of the Sgkl gene was perfectly associated with the phenotype in these mice.

3.5. Expression of Sgkl in developing hair follicles

To investigate the function of the Sgkl gene in hair development, we performed in situ hybridization of the Sgkl gene using skin sections of normal mice at different stages of hair cycle. The in situ hybridization showed no positive signal at P0 (Fig. 5A) and strong positive signals at P3, P5, and P7 (Fig. 5B, C, and D). In these stages, Sgkl mRNA was detected in the cells of the IRS, which surrounds the hair shaft and plays an important role in formation of the hair shafts. The Sgkl mRNA was also detected in a subpopulation of matrix cells at P3. Since the cells forming the IRS, which express the Sgkl gene, first appear around P3 in the normal mouse and the morphological defect in the ypc/ypc mouse also appears at P3, the expression pattern of Sgkl in the normal mouse was correlated with the appearance of the morphological defect in the mutant mice.

4. Discussion

SGKL (also termed SGK3 or CISK; Cytokine-Independent Survival Kinase) has been identified as a homolog of serum- and glucocorticoid-inducible kinase (SGK1), a serine/threonine protein kinase belonging to the AGC (protein kinase A, protein kinase G, and protein kinase C) subfamily of protein kinases. SGKs are activated by 3-phosphoinositide-dependent protein kinase-17 and play important roles in various cellular processes, including cell survival and proliferation. In particular, SGK1 is involved in the activation of SGKL, which is a serine/threonine protein kinase that plays a crucial role in the regulation of cell survival and proliferation. SGKL is activated by a variety of stimuli, including serum, glucocorticoids, and growth factors, and is known to be involved in the regulation of cell survival and proliferation. Therefore, the association between the nonsense mutation of the Sgkl gene and the morphological defect in the ypc/ypc mutant mouse is likely to be related to the activation of SGKL. Further studies are needed to clarify the exact role of SGKL in the regulation of cell survival and proliferation.
A Mutation in Sgkl Gene Causes Defective Hair Growth

**Figure 3.** Localization of the ypc locus on mouse chromosome 1. (A) Segregation of haplotypes in 1010 backcross mice obtained from crosses between YPC and JF1/Msf strains. Open and filled boxes represent homozygosity of the YPC type alleles and heterozygous F1 type, respectively. The genotypes of the ypc locus (ypc/ypc or ypc/+ ) are also denoted by the open and filled boxes. The number of backcross mice for each haplotype is indicated at the bottom of each column. (B) A partial linkage map of mouse chromosome 1 obtained in the present study and corresponding physical map obtained from the mouse genome sequence. Location of the ypc locus in relation to the linked loci is shown by an arrow. Positions of the genes, ESTs, and predicted genes are shown in the physical map with their related microsatellite markers. Distances are shown in cM and in kb on the linkage and physical maps, respectively.
kinase-1 (PDK1) upon phosphorylation of their serine/threonine residues in response to signals that stimulate phosphatidylinositol 3-kinase (PI3K).\textsuperscript{19,20} Although the functions of SGKL remain largely uncharacterized, the structure of SGKL shows significant similarity to that of protein kinase B (AKT/PKB), including an N-terminal PX or PH domain,\textsuperscript{21,22} which is responsible for the subcellular localization of the protein. AKT/PKB is the best-characterized target of phosphatidylinositol 3-kinase (PI3K) lipid products, which regulate cell growth, proliferation, survival, and differentiation.\textsuperscript{23,24} Therefore, like AKT/PKB, SGKL is believed to be involved in these cellular processes.

In the present study, we found a nonsense mutation at codon 461 of the \textit{Sgkl} gene resulting in truncation of the C-terminal 35 amino acid residues. It should be noted that the C-terminal end of the protein contains a serine residue (Ser486) that is highly conserved in the AGC subfamily of protein kinases and is phosphorylated in response to signals from PI3K.\textsuperscript{16} Nilsen et al. reported that phosphorylation of Ser486 is essential for the interaction between SGKL and PDK1,\textsuperscript{25} as mutating Ser486

---

**Figure 4.** Chromatograms showing a nucleotide substitution in the \textit{Sgkl} gene and structure of \textit{Sgkl} cDNA and SGKL. Horizontal arrows indicate the amplified fragments which cover the entire coding region of the gene. Vertical arrows in the chromatograms indicate the C-to-A nucleotide substitution at codon 461. The serine and threonine residues (Ser486 and Thr320) that are phosphorylated in response to signals from PI3K are indicated by arrowheads. Asterisks indicate the premature termination codon.

**Figure 5.** Expression of \textit{Sgkl} mRNA in developing hair follicles. A to D: \textit{In situ} hybridization with \textit{Sgkl} antisense probe. E: \textit{In situ} hybridization with \textit{Sgkl} sense probe. At P0, no signal was found (A), but strong signals were observed in the area of the IRS at P3, P5, and P7, and in part of the matrix at P3 (B, C, and D). No signal was observed in sections hybridized with sense probe (E). Bars: 50 µm.
to Ala prevents the binding of SGKL to PDK1 in vitro. Therefore, it is likely that SGKL of ypc/ypc mice lacking Ser486 cannot be activated by interacting with PDK1, leading to complete loss of its cellular function. We further revealed that the Sgkl gene is expressed specifically in particular cells of the growing hair follicles. These findings clearly indicated that the nucleotide substitution in the Sgkl gene is the causative mutation for the defective hair growth seen in ypc/ypc mutant mice. Therefore, we termed the mutant allele Sgkl<sup>Bpc</sup>.

Although the present findings suggest that SGKL is essential for proper proliferation and differentiation of the cells in hair follicles, the intracellular pathway of SGKL and its downstream molecules remain unclear. Recently, an essential role of the WNT signaling pathway in proliferation and differentiation of cells in the hair follicles has been investigated intensively.\(^3\)\(^9\) WNTs are paracrine signaling molecules that regulate cell fate determination, cell adhesive properties, and proliferation.\(^26\) WNTs initiate intracellular signaling by binding to their receptors, Frizzled (FZ),\(^1\)\(^2\) which inhibits phosphorylation of β-catenin by glycogen syntheses kinase 3 beta (GSK3β) and promotes accumulation and nuclear localization of β-catenin. In the nucleus, the accumulated β-catenin activates expression of the target genes that are required for proliferation and differentiation of hair follicles. Recently, Dai et al. reported that SGKL binds to GSK3β and phosphorylates a serine residue of GSK3β to inhibit its kinase activity.\(^27\) These findings suggested that SGKL could modify WNT signaling by inhibition of GSK3β and the lack of function of SGKL in the Sgkl<sup>Bpc</sup>/Sgkl<sup>Bpc</sup> mouse may cause degradation of β-catenin and decreased expression of the target genes in hair follicle cells. Investigation of the phosphorylation of GSK3β and accumulation of β-catenin in the cells of the mutant mice will be required to confirm this hypothesis.

The expression of the Sgkl gene has been observed in various tissue, including the lung, colon, heart, and thymus,\(^15\)\(^16\) but no apparent abnormalities were observed in these tissues in Sgkl<sup>Bpc</sup>/Sgkl<sup>Bpc</sup> mice except in the hair follicles. As the structure and function of SGKL resembles those of AKT/PKB and both kinases are regulated by PI3K signaling,\(^16\) the lack of SGKL function might be compensated by AKT/PKB or other members of the AGC subfamily of protein kinases in these tissues but not in hair follicles. It should be noted that many molecules implicated in SGKL signaling are associated with carcinogenesis. For example, AKT/PKB is the cellular counterpart of the v-Akt oncogene products,\(^28\) overexpression of the WNT gene in the mammary causes adenocarcinoma,\(^29\) APC that forms a complex with GSK3β is a product of the gene responsible for familial adenomatous polyposis (FAP),\(^30\)\(^31\) and expression of constitutively stabilized β-catenin in mouse skin results in hair follicle tumors.\(^32\) Therefore, the signaling pathway involving SGKL might be associated with particular types of carcinogenesis and Sgkl<sup>Bpc</sup>/Sgkl<sup>Bpc</sup> mice would have altered characteristics for carcinogenesis.

In the present study, we found restricted expression of the Sgkl gene in the IRS and impaired formation of the IRS in the mutant mice. The IRS surrounds the hair shafts beneath the skin surface and is composed of three morphologically distinct cell layers. The precise function of the IRS in hair morphogenesis remains unclear, but it is generally accepted that the IRS provides a supporting structure of the hair follicles, which guide appropriate formation of the hair shafts, including their uniform orientation in the skin.\(^33\) Whether the impaired IRS is the primary cause of the defective hair morphogenesis in the mutant mice is currently unclear, but the present findings demonstrated that the lack of function of SGKL in the cells of the IRS resulted in short, thin, and disorganized hair shafts as well as a lack of their uniform orientation. These findings provide new evidence to support the functional importance of the IRS in hair morphogenesis. Several genes for transcription factors, including Gata3,\(^34\) Cutl1,\(^35\) and hp36 have been identified as having particular functions in the IRS. For example, expression of the Gata3 gene in hair follicles is highly restricted to the epidermis and IRS and mice with knockout mutations in the Gata3 gene showed that IRS progenitors failed to differentiate and IRS could not be formed in the hair follicles.\(^34\) Therefore, GATA-3 is believed to be a key factor for cell fate determination of IRS progenitor cells. Further investigations of the function of SGKL as well as the relationship between SGKL and these transcription factors in the formation of IRS will provide new insight into the function of the IRS in hair follicle morphogenesis.

References

1. Fuchs, E., Merrill, B. J., Jamora, C., and DasGupta, R. 2001, At the roots of a never-ending cycle, Dev. Cell, 1, 13–25.
2. Kishimoto, J., Burgeson, R. E., and Morgan, B. A. 2000, Wnt signaling maintains the hair-inducing activity of the dermal papilla, Genes Dev., 14, 1181–1185.
3. Millar, S. E., Willert, K., Salinas, P. C. et al. 1999, Wnt signaling in the control of hair growth and structure, Dev. Biol., 207, 133–149.
4. Mann, G. B., Fowler, K. J., Gabriel, A., Nice, E. C., Williams R. L., and Dunn, A. R. 1993, Mice with a null mutation of the TGFβ gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation, Cell, 73, 249–261.
5. Luetteke, N. C., Qiu, T. H., Peiffer, R. L., Olicer, P., Smithies, O., and Lee, D. C. 1993, TGFβ deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice, Cell, 73, 263–278.
6. Kobielak, K., Pasolli, H. A., Alonso, L., Polak, L., and Fuchs, E. 2003, Defining BMP functions in the hair follicle by conditional ablation of BMP receptor IA, J. Cell
21. Xu, J., Liu, D., Gill, G., and Songyang, Z. 2001, Regulation of cytokine-independent survival kinase (CISK) by the Phox homology domain and phosphoinositides, J. Cell Biol., 154, 699–705.

22. Virbasius, J. V., Song, X., Pomerleau, D. P., Zhan, Y., Zhou, G. W., and Czech, M. P. 2001, Activation of the Akt-related cytokine-independent survival kinase requires interaction of its phox domain with endosomal phosphatidylinositol 3-phosphate, Proc. Natl. Acad. Sci. USA, 98, 12908–12913.

23. Chan, T. O., Rittenhouse, S. E., and Tsichlis, P. N. 1999, AKT/PKB and other D3 phosphoinositide-regulated kinases: kinase activation by phosphoinositide-dependent phosphorylation, Annu. Rev. Biochem., 68, 965–1014.

24. Downward, J. 1998, Mechanisms and consequences of activation of protein kinase B/Akt, Curr. Opin. Cell Biol., 10, 262–267.

25. Nilsen, T., Slagsvold, T., Skjerpen, C. S., Brech, A., Stenmark, H., and Ohnes, S. 2004, Peroxisomal targeting as a tool for assay ing protein-protein interactions in the living cell: cytokine-independent survival kinase (CISK) binds PDK-1 in vivo in a phosphorylation-dependent manner, J. Biol. Chem., 279, 4794–4801.

26. Dale, T. C. 1998, Signal transduction by the Wnt family of ligands, Biochem. J., 329, 209–223.