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Expression of Mineralized Tissue Associated Proteins: Dentin Sialoprotein and Phosphophoryn in Rodent Hair Follicles

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Abstract

Background—Mammalian hair development and tooth development are controlled by a series of reciprocal epithelial-mesenchymal interactions. Similar growth factors and transcription factors, such as fibroblast growth factor (FGF), sonic hedgehog homolog (SHH), bone morphogenetic proteins (BMPs) and Wnt10a, were reported to be involved in both of these interactions. Dentin sialoprotein (DSP) and phosphophoryn (PP) are the two major non-collagenous proteins secreted by odontoblasts that participate in dentin mineralization during tooth development. Because of striking similarities between tooth development and hair follicle development, we investigated whether DSP and/or PP proteins may also play a role in hair follicle development.

Objective—In this study, we examined the presence and location of DSP/PP proteins during hair follicle development.

Methods—Rat PP proteins were detected using immunohistochemical/immunofluorescent staining. DSP-PP mRNAs were detected by *in situ* hybridization with riboprobes. LacZ expression was detected in mouse tissues using a DSP-PP promoter-driven LUC in transgenic mice.

Results—We found that PP proteins and DSP-PP mRNAs are present in rat hair follicles. We also demonstrate that an 8 kb DSP-PP promoter is able to drive lacZ expression in hair follicles.

Conclusion—We have firmly established the presence of DSP/PP in mouse and rat hair follicles by immunohistochemical/immunofluorescent staining, in situ hybridization with riboprobes and transgenic mice studies. The expression of DSP/PP in hair follicles is the first demonstration that major mineralization proteins likely may also contribute to soft tissue development. This finding

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opens a new avenue for future investigations into the molecular-genetic management of soft tissue development.

Keywords

Dentin protein expression; dentin sialoprotein; phosphophoryn; hair follicles

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1. Introduction

Mammalian hair development is controlled by a series of reciprocal interactions between epithelium-derived pluripotent matrix cells and mesoderm-derived dermal papilla cells [1]. The pluripotent epithelial matrix cells at the base of hair follicles are derived from the long-lived stem cells located at the permanent, bulge region of the follicle. These epithelial matrix cells can give rise to different hair follicle lineages including the medulla/cortex/cuticle of the hair shaft and the cuticle/Huxley's layer/Henle's layer of inner root sheath [2]. Various growth factors and transcription factors, such as fibroblast growth factor (FGF), sonic hedgehog homolog (SHH), bone morphogenetic proteins (BMPs) and Wnt10a, were reported to be involved in these interactions during hair follicle development [3, 4]. In a strikingly similar way, tooth development is also controlled by a series of reciprocal epithelial-mesenchymal interactions involving FGF, SHH, BMPs and Wnt10a [1]. In this case, a single layer of polarized odontoblasts secrete an un-calcified pre-dentin matrix comprised mainly of collagen type I and non-collagenous proteins (NCPs) which causes the conversion of un-calcified pre-dentin to calcified dentin. Studies on a mutated BMP receptor 1A by Andl and co-workers [5] demonstrated that tooth morphogenesis was arrested and hair follicle development was defective. This work suggested that both hair development and tooth development may be under the control of similar factors.

Two important dentin NCPs, dentin sialoprotein (DSP) and phosphophoryn (PP) (encoded within a single odontoblast-specific DSP-PP transcript), are secreted at the mineralization front via odontoblastic processes [6–9]. Their appearance is coupled to the conversion of un-calcified pre-dentin to calcified dentin, and PP in particular is believed to participate in the initiation and control of hydroxyapatite formation since DSP-PP knockout mice exhibit tooth defects similar to human dentinogenesis imperfecta III with enlarged chambers, increased width of the pre-dentin zone, hypomineralization and pulp exposure [10]. Transgenic mice studies from our laboratory showed that DSP-PP promoter-directed lacZ expression was found in tooth, bone, and kidney tissues, suggesting the possibility of a broad range of DSP/PP biological activities beyond dentin mineralization [11].

Because of striking similarities between tooth development and hair follicle development, we began to investigate whether DSP or PP proteins may also play a role in hair follicle development. We found that PP proteins and DSP-PP mRNAs are present in rat hair follicle by immunohistochemistry staining and *in situ* hybridization with riboprobes. We also demonstrate that an 8 kb DSP-PP promoter is able to drive lacZ expression in hair follicles. Furthermore, we demonstrate that two other mineralized tissue proteins, osteopontin (OPN) and bone sialoprotein (BSP), are present in hair follicles.

2. Materials and methods

2.1 Animals and tissues preparation

Post-natal, 3-day-old, 14-day-old, 5-month-old Sprague Dawley and 5-week-old Long Evans rats were euthanized. Heads were dissected and perfused with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) for 2d at 4°C, followed by decalcification in 8% ethylenediaminetetraacetic acid (EDTA) (pH 7.4) at 4°C for 1–5wk. All tissues were

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processed, embedded in paraffin, and sectioned (5 μm) for immunohistochemistry and *in situ* hybridization. Some of the sections were stained by hematoxylin and eosin (H&E) for general histological details.

The 8 kb rat promoter DSP-PP-LacZ transgene mice were obtained as previously described by our group [11]. Animals were euthanized at post-natal, 3-day-old, and 5-day-old, and were processed for cryostat sections. Fresh samples from transgenic whole embryos, neonatal mice and post-natal head were processed by cryosection (12 μm) for galactosidase assay and *in situ* hybridization. This work was done under University of Michigan UCUCA protocol #7756A for Transgenic Core.

2.2 Immunohistochemistry

Rabbit anti-rat PP antibodies: Rat highly-phosphorylated protein (HP) was isolated from rat incisor dentin by the method of Marsh [31]. HP contained 2.9 nmol Pi/μg HP. 1 mg of HP was conjugated to KLH and injected into rabbits to generate anti-rat PP antibodies. The rabbits were immunized four times over a span of 2 ½ months, test bled and EIA assayed using HP-immobilized microtiter wells, followed by a total bleed after the final boost. Anti-PP antibodies diluted 1:500 were able to specifically detect purified HP protein and to detect HP in dentin extract.

Monoclonal mouse anti-rat bone sialoprotein (BSP) antibodies and osteopontin (OPN) were purchased from the Developmental Studies Hybridoma Bank at the University of Iowa (Iowa City, Iowa, USA).

Paraffin-embedded sections were deparaffinized in xylene, rehydrated in graded ethanols and then in phosphate-buffered saline (PBS). Samples were washed three times in PBS. Briefly, sections were incubated for 30 minutes in diluted blocking serum, then incubated at 4°C overnight with the primary antibodies to PP, BSP and OPN at dilutions of 1: 500, 1: 200, and 1:200, respectively. After rinsing in PBS for 5 minutes, sections were treated with pre-diluted alkaline phosphatase-conjugated secondary antibody for 30 minutes and then washed three times in PBS. For alkaline phosphatase-based antibody detection, endogenous alkaline phosphates was suppressed by incubation in 0.24 mg/ml levamisole for 30 minutes. The immunostaining reaction was next developed in detection solution (for every 10 ml of the substrate solution, add 200 ul of NBT/BCIP pre-made solution (Roche, Indianapolis, IN)) for 10 minutes. Sections were then cleared and mounted. Control sections were treated with normal rabbit immunoglobulin, rather than a specific primary antibody using the same immunoglobulin concentration.

2.3 Immunofluorescence staining

For immunofluorescence staining, sections were treated with Pepsin Solution for Enzyme-induced Epitope Retrieval (Thermo Scientific, Fremont, CA) at room temperature for 15 minutes and washed in PBS for 2 × 5 minutes, followed by a 30-minute incubation in diluted blocking serum. They were then incubated at 4°C overnight with the following three antibodies: (1) the primary rabbit antibodies to rat PP, (2) primary guinea pig antibodies to human keratin K71 (Fitzgerald Industries International, Inc. Concord, MA) and (3) primary rabbit anti-human K75 (Sigma-Aldrich, St. Louis, MO) at dilutions of 1: 500, 1: 100, and 1:50, respectively. After rinsing in PBS three times for 5 minutes, sections were treated with Texas Red dye-conjugated goat anti-rabbit IgG secondary antibodies (for anti-PP staining), FITC-conjugated goat anti-guinea pig IgG secondary antibodies (for anti-K71 staining) and Texas Red dye-conjugated rabbit anti-goat secondary antibodies (for anti-K75 staining), for 2 hours and then washed three times with PBS. The samples were mounted and observed under a UV microscope and photographs were taken.

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2.4 Galactosidase assay

LacZ activity was detected by the staining of frozen sections from DSP-PP-LacZ transgene mice with X-gal (5-bromo-4-chloro-3-indolyl-galactopyranoside; Boehringer-Mannheim) [11]. Slides were fixed for 5 minutes with 0.2% glutaraldehyde, 5 mM EGTA, 2 mM $MgCl_2$ in 0.1 M sodium phosphate buffer at pH 7.3. They were then washed three times for 5 minutes each with a rinse solution containing 2 mM $MgCl_2$, 0.2% NP-40, and 0.1% sodium deoxycholate in 0.1 M sodium phosphate buffer at pH 7.3. Staining was performed in the dark at 37°C overnight in the rinse solution supplemented with 1 mg/ml X-gal, 5 mM potassium ferrocyanide, and 5 mM potassium ferricyanide. After staining, the slides were washed with PBS and counterstained with 1% neutral red or 1% eosin to indicate morphology.

2.5 In situ hybridization

Templates for anti-sense and sense riboprobes for the DSP-PP gene were generated by digesting DSP-PP_{523} cDNA (which codes for DSP protein and a 523 amino acid PP isoform) with appropriate restriction enzymes and an *in vitro* transcription assay was carried out to incorporate digoxigenin-11-dUTR with T7 and Sp6 RNA polymerases according to standard manufacturer's protocol (Roche Diagnostics, Indianapolis, IN). De-waxed paraffin sections were fixed with 4% paraformaldehyde (PFA) in PBS buffer. After treatment with proteinase K (10 μg /ml) for 10 minutes, the paraffin sections were postfixed in 4% PFA for 5 minutes and dipped in 0.1 M triethanolamine containing 0.25% acetic anhydride for 15 minutes. Hybridization was performed at 50°C using denatured anti-sense or sense riboprobes in hybridization buffer (40% formamide, 5×SSC, 1×Denhardt's solution, 100 μg /ml salmon DNA, 100 μg/ml yeast tRNA) for 20 hours. After post-hybridization treatments, including RNase A treatment (10 μg/ml, 24 minutes, 37°C) followed by thorough washes, the sections were reacted with anti-digoxigenin antibody conjugated with alkaline phosphatase (1:1000) and stained with NTB/BCIP solution. Sections were observed and photographed with a Nikon Eclipse E400 light microscope and a Spot Real Time camera.

3. Results

3.1 Phosphophoryn protein is present in rat hair follicles

We first examined whether PP protein is present in rat hair follicles. Using an anti-rat PP antibody, PP proteins were detected in the hair follicles from 5-week-old Long Evans (LE) rats and 5-month-old Sprague Dawley (SD) rats (Figure 1). Furthermore, PP proteins were detected in the sebaceous gland and the epidermis layer (Figure 1).

To further examine the location of PP protein expression in hair follicles, we used anti-K71 antibodies, a marker for the inner root sheath [12], and anti-K75 antibodies, a marker for the companion layer and medulla [12], to establish the relative positions of PP protein expression within the hair follicles. Using anti-K71 antibodies, we detected the inner root sheath in longitudinal paraffin-embedded sections obtained from 14-day-old SD rat hair follicles (Figure 2A) and in cross-sections obtained from 3-day-old SD rat hair follicles (Figure 2B). Using FITC-conjugated secondary antibodies, anti-K75 antibodies detected the keratin K75 marker in the companion layer and in the hair follicle medulla in longitudinal sections obtained from 14-day-old SD rats (Figure 2C) and in the companion layer and in medulla in cross sections of 3-day-old SD rat hair follicles (Figure 2D). Using Texas-Red-conjugated secondary antibodies, anti-PP antibodies demonstrated that PP protein was expressed in the outer root sheath and matrix cells in the longitudinal sections obtained from 14-day-old hair follicles (Figure 2E) and in the outer root sheath in cross sections of 3-day-old hair follicles.

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3.2 Osteopontin (OPN) and bone sialoprotein (BSP) are present in rat hair follicles

The detection of PP protein in hair follicles (see Figure 1) prompted us to test whether other mineralizing tissue proteins, such as osteopontin (OPN) and bone sialoprotein (BSP), were also present in hair follicles. Using anti-OPN and anti-BSP antibodies, both OPN and BSP were detected in hair follicle samples from 5-week-old Long Evans rats and 5-month-old Sprague Dawley rats (Figure 3).

3.3 In situ hybridization demonstrates the presence of DSP-PP$_{523}$ mRNA in hair follicles

PP protein is expressed in rat hair follicles (see Figure 1). To further validate this finding, a tissue section from post-natal 3-day-old Sprague Dawley rat skin tissue was subjected to *in situ* hybridization using a rat digoxigenin (DIG)-labeled DSP–PP$_{523}$ anti-sense riboprobe. Figure. 4A–B represents light-field photographs of longitudinally-sectioned 5-month-old Sprague Dawley rat hair follicles. Figure 4D clearly demonstrates DIG-staining of the anti-sense DSP-PP$_{523}$ riboprobe in 3-day-old rat hair follicles. This rat anti-sense riboprobe result further confirms our immunohistochemical PP staining results by demonstrating that DSP-PP mRNA is expressed in the hair follicles. No DIG staining was observed with the sense riboprobe (Figure 4C).

3.4 An 8 kb DSP-PP promoter drives lacZ expression in hair follicle

Previously, a rat DSP-PP construct, comprising a −5 kb upstream DSP–PP promoter, an exon1–intron1 sequence, and a LacZ gene construct (hereinafter ‘‘8 kb rat promoter’’), was used to test DSP–PP promoter-directed LacZ activity in transgenic mice [11]. The 8 kb promoter-driven LacZ tissue expression patterns were found to mimic many of the endogenous DSP–PP expression patterns that were earlier reported in rat teeth [8] and in mouse teeth [13]. Thus, this 8 kb promoter contains sufficient cis-elements to ensure correct temporal and spatial expression patterns in teeth. To test whether this 8 kb promoter contains sufficient cis-elements to drive lacZ expression in hair follicles, frozen sections from DSP-PP-LacZ transgenic mice were stained with X-gal. As shown in Figure 5A, this 8 kb DSP-PP promoter does drive lacZ expression in the hair follicles in post-natal 3-day-old transgenic mice and in the hair follicles obtained from 5-day-old transgenic mice (Figure 5B). Thus, this 8 kb DSP-PP promoter also contains sufficient cis-elements for lacZ expression in hair follicles.

Taken together, we have firmly established that PP protein expression and DSP-PP mRNA expression occurs in the hair follicles. We have shown that the DSP-PP promoter controlling correct lacZ expression patterns in tooth and bone also controls lacZ expression in hair follicles.

4. Discussion

BSP, OPN, DSP and PP all belong to the family of small integrin-binding ligand N-linked glycoproteins (SIBLINGs) [14]. BSP and PP act as nucleators that drive mineralization in tissues such as bone and dentin [15, 16]. OPN can inhibit apatite crystal growth in bone and dentin [15, 16]. DSP was also reported as a weak inhibitor for apatite formation [17]. DSP/PP are the dominant non-collagenous proteins in dentin. Here, we report the expression of PP protein in the outer root sheath (ORS) and in the matrix cells of rat hair follicles obtained from 5-week-old Long Evans (LE) and 5-month-old Sprague Dawley (SD) rats. We also report the expression of PP protein in the matrix cells (Figures 1 and 2). In addition, we detected PP protein expression in sebaceous glands and the epidermis layer of 5-week-old LE rats and 5 month-old SD rats. Using *in situ* techniques, we detected the expression of DSP-PP mRNA in hair follicles and using an 8 kb DSP-PP promoter driven LacZ transgenic mouse, we found LacZ expression in hair follicles. Furthermore, using immunolocalization,

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we also demonstrated BSP and OPN expression in hair follicles. The expression of all of these mineralizing tissue marker proteins in hair follicles suggests that these proteins may significantly contribute to hair follicle development.

Since these proteins are well known to be associated with calcium deposition in bone and dentin leading to tissue mineralization, we speculate that DSP, PP, OPN and BSP might contribute to calcium deposition, which strengthens the hardness of the hair root sheath. This possibility is supported by the findings of Pearce et al [18], who used direct chemical and X-ray diffraction to demonstrate that hair follicles are susceptible to mineralization when the skin of hypercalcaemic rats is injured. The X-ray diffraction image of the hair follicles in the injured region showed a diffuse apatite pattern. In another study, tumors obtained from patients with the disease pilomatrioma showed distinct microscopic resemblance to calcifying odontogenic cysts [19]. In this disease, the calcifying epithelioma of Malherbe is believed to originate from hair follicle matrix cells. The detection of PP protein in matrix cells (Figure 2E) suggests that PP might contribute to the calcification of epithelioma.

Trichohyaline is a 190 kDa protein with a high content of lysine and glutamic acid/glutamine residues and is rich in arginine residues [20]. Steinert and co-workers have suggested that trichohyaline may provide mechanical strength to hair follicles due to multiple cross-bridging roles in the inner root sheath [21]. It would be interesting to test whether DSP/PP, OPN and BSP can contribute to root sheath hardness in order to further strengthen the root sheath.

Recently, we reported that PP isoform PP_{523} (containing 523 amino acids) exhibits proteolytic activity toward gelatin [22]. DSP-PP promoter-driven LacZ expression appears in the kidneys of post-natal 3-day-old transgenic mice [11], in alveolar bone in newborn mice prior to its appearance in the incisor, and in the salivary glands obtained from newborn mice (Ritchie, unpublished data). The newly found proteolytic activity in PP proteins suggests that PP proteins may play key role in tissue modeling. The finding that PP protein, as well as DSP-PP transcript expression and DSP-PP promoter-driven LacZ expression all occur in hair follicles, supports a possible role of PP proteins in tissue modeling during hair follicle development. BSP and OPN were reported to tightly bind to and activate pro-MMP-2 and pro-MMP-3, respectively [14], thus they may also have a role in tissue remodeling during hair follicle development.

As mentioned above, our 8 kb DSP-PP promoter is able to drive lacZ expression in teeth and in hair follicles. A number of transcription factors, such as FGFs, BMP4, SHH, and Wnt10a, contribute to tooth and hair follicle development and may directly or indirectly contribute to this 8 kb DSP-PP promoter to enable DSP-PP expression in both teeth and hair follicles. Thus, there may be common developmental pathways present in teeth and in hair follicles such that any mutation or deficiency of genes in these pathways would likely affect both tooth germ and hair follicle development. This idea is supported by the findings that (1) BMP7 expression occurs in tooth germs [23] and in hair follicles [24], (2) BMP-r1A mutation resulted in arrested tooth morphogenesis as well as defective hair follicle development [5], (3) Cbfa1/Runx2, a transcription factor, is known to control skeletal and tooth development [25–27], and tooth organs in Cbfa1/Runx2-null mice lacked overt odontoblast and ameloblast differentiation, as well as normal dentin and enamel development [25]. Runx2-deficient mice exhibited impaired skin and hair follicle development [28], and (4) the expression of Dickkopf I, a potent diffusible inhibitor of Wnt action, blocked both hair and tooth development [29]. Research directed toward these transcription factors or the interaction of their downstream products with the 8 kb DSP-PP promoter may provide further insights into our understanding of common developmental pathways leading to tooth and hair follicle development. Finally, the presence of common

developmental pathways between teeth and hair follicles may be responsible for the abnormal hair and teeth observed in X-linked anhidrotic ectodermal dysplasia (EDA) [30] and the observed kinky curly hair, thin-pitted enamel and taurodontism in tricho-dento-osseous (TDO).

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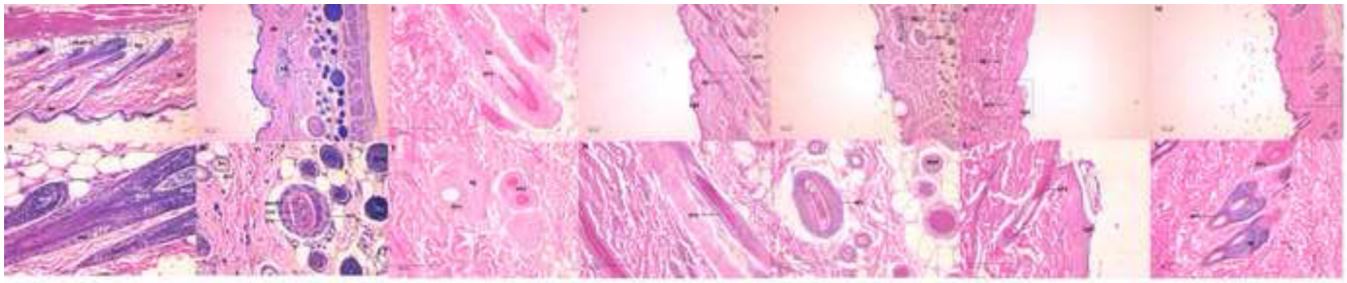

Figure 1. Hematoxylin & Eosin (H&E) staining and PP protein expression in hair follicles from different hair cycles

Using highly specific anti-PP antibodies, immunohistochemical (IHC) staining was performed in the hair follicles of 5-week-old Long Evans (LE) rats and 5-month-old Sprague Dawley (SD) rats. A–D represent Hematoxylin & Eosin staining of dorsal skin hair follicles at growth hair cycle stage (i.e., anagen and catagen). The longitudinal section of hair follicles from 5-month-old SD rats at 100× and 400× magnification are presented in A and B, and the cross sections are in C and D. E–F: Negative control for IHC, SD hair follicle sections were treated with normal goat immunoglobulin instead of anti-PP antibodies. No staining was detected (400× magnification). G–J: Anti-PP antibodies were used to detect PP proteins in hair follicles at the growth phase. G and H represent the longitudinal section of 5-month-old SD rat hair follicles at 100× and 400× magnification. I and J represent the cross section of 5-week-old LE rat hair follicles at 100× and 400× magnification. PP proteins are expressed in the hair follicle outer root sheath, matrix cells, sebaceous glands and the epidermis layer. Positive staining is blue, as indicated by arrows. K-N: Anti-PP antibodies were used to detect PP proteins in 5-month-old SD hair follicles at the resting phase. In telophase of the SD hair follicle, PP proteins were expressed in the outer root sheath and epidermis layer (K,L) at 100× and 400× magnification. PP expression is very strong in sebaceous glands (M,N) at 100× and 400× magnification. Abbreviations: epi, epidermis; de, dermis; hs, hair shaft; sg, sebaceous gland; P, papilla; Mat, hair matrix; Med, hair medulla; IRS, inner root sheath; ORS, outer root sheath. The scale bar represents 100 μm.

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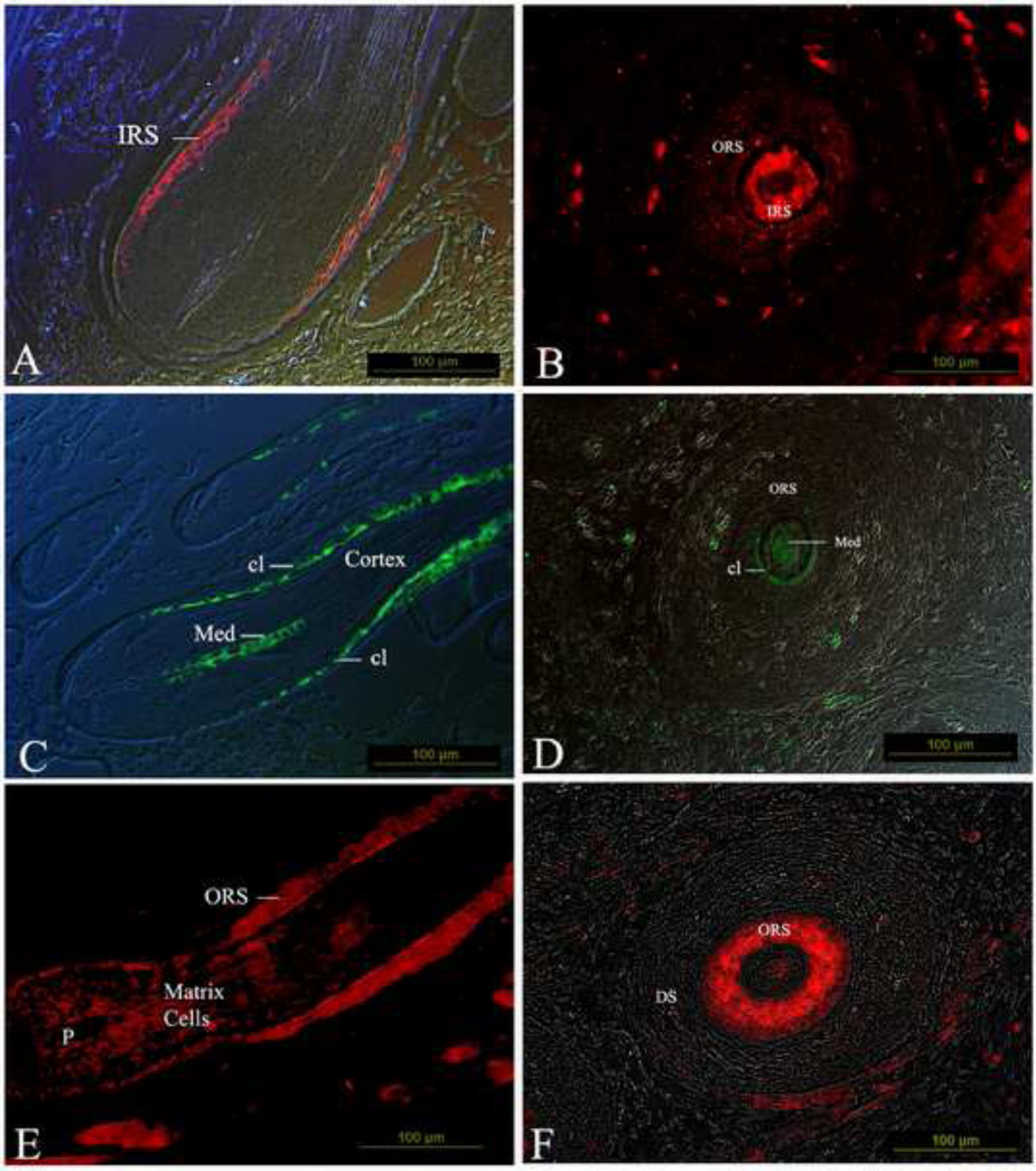

Figure 2. Immunofluorescent detection of keratin K71, keratin K75 and phosphophoryn in hair follicles

We used anti-K71 and anti-K75 primary antibodies with the appropriate immunofluorescent secondary antibodies (see section 2.3) to establish the relative position of inner root sheath, companion layer and medulla. Similarly, we used anti-PP primary antibodies with the appropriate secondary antibodies to perform immunofluoresence to determine the location of PP expression within the hair follicles. A: Anti-K71 antibodies with Texas Red-conjugated secondary antibodies recognized the inner root sheath (IRS) (longitudinal section). B: Anti-K71 antibodies with Texas Red-conjugated secondary antibodies recognized the inner root sheath (cross section). C: Anti-K75 antibodies with FITC-

conjugated secondary antibodies recognized the companion layer (cl) and medulla (Med) (longitudinal section). D: Anti-K75 antibodies with FITC-conjugated secondary antibodies recognized the cl and med (cross section). E: Anti-PP antibodies with Texas Red-conjugated secondary antibodies recognized the outer root sheath (ORS) and matrix cells (longitudinal section). F: Anti-PP antibodies with Texas Red-conjugated secondary antibodies recognized the outer root sheath. P, papilla. DS, dermal sheath. The scale bar represents 100 μm.

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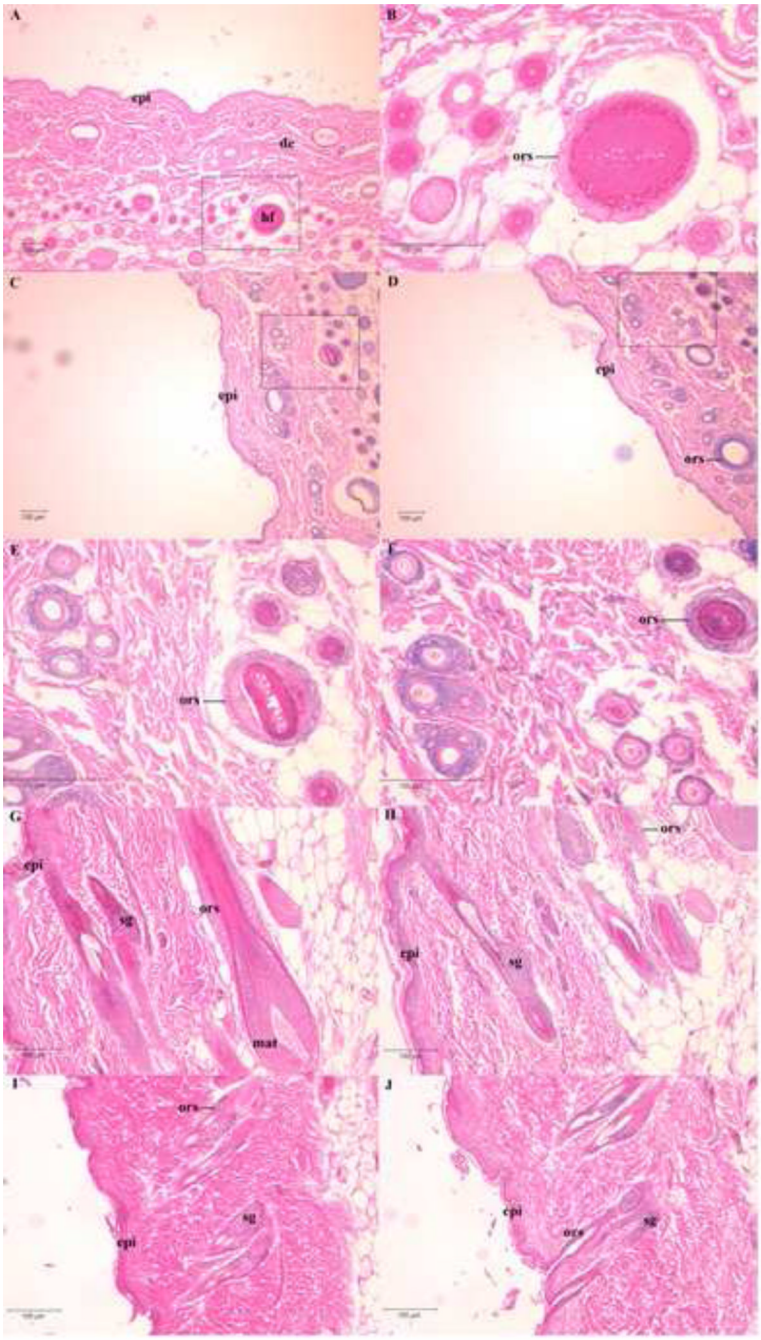

Figure 3. Detection of osteopontin (OPN) and bone sialoprotein (BSP) in the root sheath of hair follicles

Using normal goat serum as primary antibodies, no staining was detected in the control group (A,B). Using immunohistochemistry staining with anti-BSP antibodies to examine the follicles at the growth phase (C,E,G) and resting phase (I), BSP was detected in the outer root sheath (ORS), sebaceous gland and epidermis layer at 100×, 200× and 400× magnifications (C,E,G,I). Using immunohistochemistry staining with anti-OPN antibodies to examine the follicles at the growth phase (D,F,H) and resting phase (J), OPN was detected in the outer root sheath (ORS), sebaceous gland and epidermis layer at 100×, 200× and 400× magnification (D,F,H,J). Blue indicates the positive staining, as shown by arrows.

A-F are from 5-week-old LE rats. G-J are from 5-month-old SD rats. The scale bar represents 100 μm.

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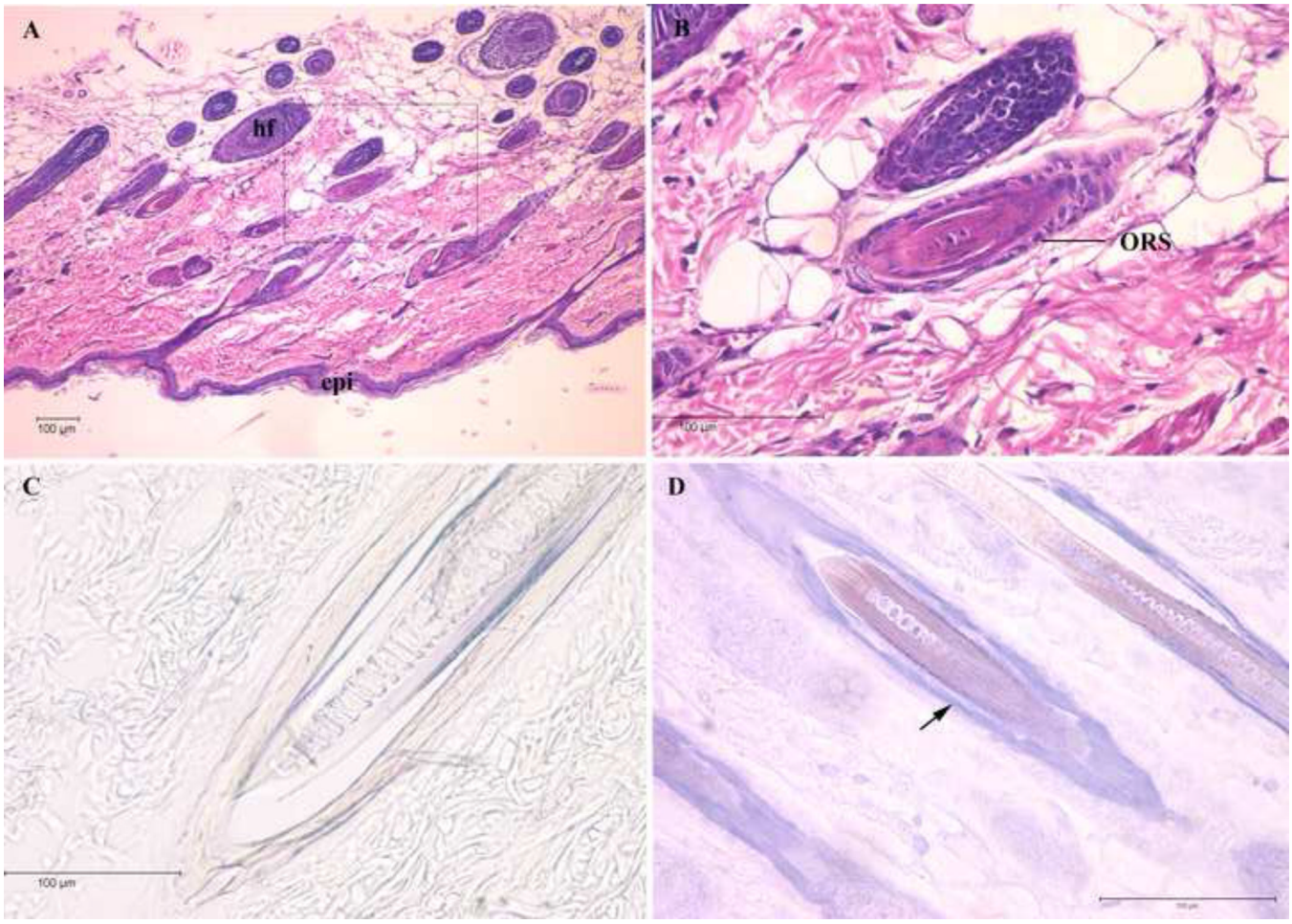

Figure 4. Detection of DSP-PP$_{523}$ mRNA in the hair follicles using *in situ* hybridization

A–B: A light field photograph of 5-month-old SD rat hair follicles at the longitudinal section at 100× and 400× magnifications. C: No DIG-staining was observed with the sense riboprobe. D: Using a DIG-labeled anti-sense DSP-PP$_{523}$ riboprobe to perform *in situ* hybridization, blue staining (indicated by an arrow) was detected in hair follicles from 3-day-old SD rat at 400× magnification. The scale bar represents 100 μm.

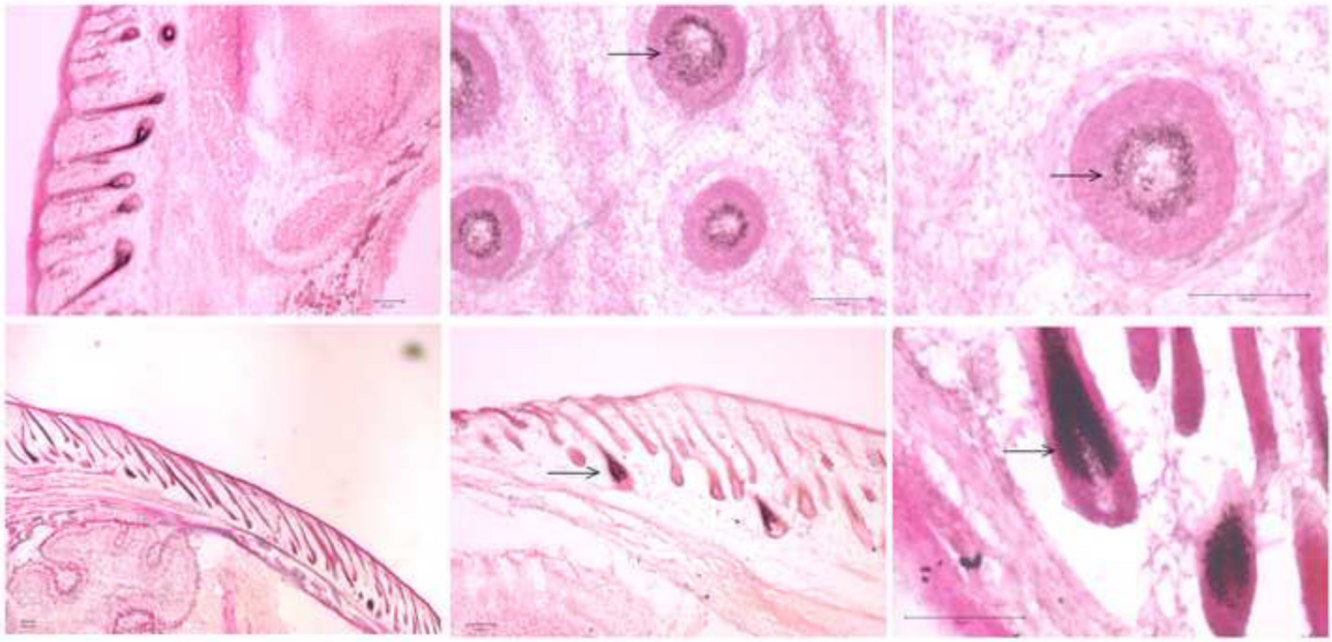

Figure 5. An 8 kb DSP-PP promoter drives lacZ expression in the inner root sheath in transgenic mice

A rat 8 kb DSP-PP promoter linked to lacZ was used to generate transgenic mice. Sections from 3-day-old (A–C) and 5-day-old (D–F) transgenic mice containing this 8 kb DSP-PP promoter-lacZ were assayed for lacZ activity. A: LacZ expression in hair follicles of 3-day-old transgenic mice (longitudinal sections; 100× magnification). B and C: LacZ expression in hair follicles of 3-day-old transgenic mice (cross-sections; 100× and 200× magnification, respectively). D and E: LacZ expression in the hair follicles of 5-day-old transgenic mice (longitudinal sections; 40× and 100× magnification, respectively). F: LacZ expression in hair follicles of 5-day-old transgenic mice (cross section; 400× magnification). LacZ expression is indicated by arrows. The scale bar represents 100 μm.

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