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Research Article

Investigation of Insulin-Like Growth Factor 1 Receptor Expression in Sacrococcygeal Pilonidal Sinus

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Abstract

Aim: This study aims to determine the role of IGF-1R in the sacrococcygeal pilonidal sinus (PS) etiology and evaluate the findings regarding its contribution to treatment.

Material and Methods: The general structure of skin and connective tissue components in healthy and lesioned tissue sections of sacrococcygeal PS cases was evaluated by Masson's trichrome staining. In addition, the expression of IGF-1R protein in healthy and pilonidal sinus tissue was determined by immunohistochemical staining.

Results: It was observed that the epidermis of the pilonidal sinus was thinned compared to the healthy area, and the hair follicle structures and connective tissue components deteriorated. IGF-1R expression was significantly decreased in basal keratinocytes in sacrococcygeal PS tissues.

Conclusion: It is thought that IGF-1R may be involved in the etiology of sacrococcygeal PS, and more data is needed in terms of its contribution to treatment.

Keywords: Sacrococcygeal pilonidal sinus, IGF-1R, keratinocytes

INTRODUCTION

Pilonidal sinus is a relatively common dermatological soft tissue disease that affects both the pediatric population and adults (1). It is twice as common in men as in women and is a suppurative condition that usually occurs between the ages of 15 and 30. The pilonidal sinus is formed under the skin of the sacrococcygeal region, and the acute form presents as a tension-generating abscess, while the chronic condition causes intermittent discharge (2). The disease, which is common among young people, significantly affects the quality of life and causes low self-esteem. Despite its partially known pathophysiology and the numerous treatment modalities available, pilonidal cysts still pose a significant problem in general surgery (3).

The pilonidal sinus originates from a congenital skin cleft caused by hair growth (4). The skin covering the sacral pilonidal sinus contains unusually deep hairy papillae. Although the follicles are separate from the sinus, they may be abnormally large, and each may carry more than one hair. None of these features are observed in the sacral skin of normal controls. Many factors affect the hair follicle's growth, and systemic factors such as hormones and environmental factors are some of them (5). A healthy skin and follicle structure (Figure 1/a) is also schematized. Although factors such as BMP, Wnt, and FGF are thought to be effective in hair growth (6), literature information on the role of insulin-like growth factors is negligible.

Epidermal stem cells are keratinocytes located in the basal layer of the epidermis and mediate epidermal homeostasis. Signaling through the insulin-like growth factor (IGF-1) receptor (IGF-1R), IGF-1 has been identified as an essential regulator of rodent skin development and differentiation. However, the role of IGF-1/IGF-1R

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signaling in human keratinocytes is not fully understood. In epithelialization models using human keratinocytes in three-dimensional cultures, it has been observed that loss of IGF-1/IGF-1R signaling causes decreased skin thickness and impaired stem cell homeostasis (7). Loesch et al. demonstrated that IGF-1R controls DNA damage repair genes in human keratinocytes (8).

Although information on skin structure and related skin diseases can be found in the literature regarding IGF-1 and IGF-1R, no data has been found regarding its role in the development of pilonidal sinus. Therefore, while planning our study, we thought that an evaluation of the histopathology of the disease would provide information for future studies on this subject and add new data to the literature. We hypothesized that IGF-1R could also be involved in the etiology of pilonidal sinus and show changes at the tissue level, mainly based on the accepted assumptions on this subject.

MATERIAL AND METHOD

Tissue Preparation

Between 2012 and 2018, sacrococcygeal pilonidal sinus cases were diagnosed with hematoxylin-eosin staining from the excisional skin tissues admitted to the Pathology Laboratory of Bozok University Hospital were evaluated in terms of material adequacy, and tissues with and without lesions were obtained. The experimental groups of our study; Group 1 (n=10): Lesioned regions of sacrococcygeal pilonidal sinus cases diagnosed by excisional skin biopsies, Group 2 (n=10): Tissues from non-lesional areas obtained from excisional skin biopsies of the same patients were formed. The study was initiated with the permission of the Akdeniz University Clinical Research Ethics Committee, numbered 08.03.2023/KAEK-198.

Sections of 5 µm thickness were taken with a microtome from healthy and lesionless areas of sacrococcygeal pilonidal sinus cases and embedded in paraffin. They were placed on a superfrost slide and kept in an oven at 56°C overnight to apply the immunohistochemical staining method.

Immunohistochemical Staining

Sections were rehydrated by passing through xylol and alcohol series (100%, 90%, 80%, 70%) for deparaffinization. To remove antigenic masking, it was boiled in citrate buffer (100244; Merck) and incubated with hydrogen peroxide (18312; Sigma) to remove endogenous peroxidase activity. Sections treated with UV blocking (TA-125-UB Thermo Scientific) to prevent non-specific immunoglobulin binding were incubated with IGF-1R primary antibody (Biocare; sku 414-110917) and appropriate secondary antibody (Vector anti Mouse; BA-9200). After making the reaction visible with DAB chromogen (D4168; Sigma) and counterstaining the sections with Mayer's Hematoxylin (109249, Merck), dehydrated sections were passed through alcohol series (70%, 80%, 90%, 100%), and xylol. After that closed with entellan. In these sections, the expression levels and localizations of the IGF-1R protein in the intact and lesioned tissues of the patients were tried to be determined. In addition, the tissues were subjected to Masson's trichrome staining, a unique connective tissue stain, to make histopathological evaluations between the groups and examine the connective tissue changes. Sections taken on the slide were kept in an oven at 56°C overnight, then deparaffinized and stained following the protocol of Masson's trichrome staining kit (GBL-5022). Sections were passed through an alcohol series and xylol for dehydration and then covered with entellan. Sections by photographing the Olympus CX43 Microscope (Japan) to visualize the localization of the IGF-1R protein.

H-Score Analysing

The marker's immunohistochemical staining power was examined with Histoscore (H-score). H-score was calculated by a semi-quantitative assessment of both the intensity of staining (graded as non-staining; -/+, weak; +, median; ++, strong; +++) using adjacent normal mucosa as the median and the percentage of the positive cells.

Statistical Analysis

The staining of six sections taken for all subjects in each group was repeated three times. Six different areas were photographed on three randomly selected slides for each subject and measured with Image J (1.52 R, National Institutes of Health, USA). The measurements were evaluated with GraphPad Prism 9 (GraphPad Software, USA) using the student t-test. The difference between the groups ($p<0.05$) was considered significant. The drawings in Figure 1 were made using the BioRender medical illustration program.

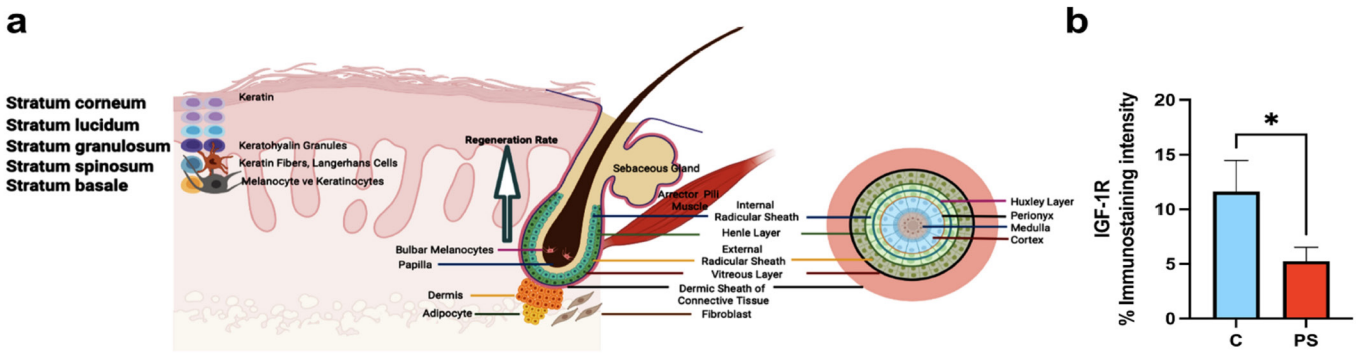

Figure 1. a: Drawing showing a healthy epidermis and the layers of the hair structure and the cells they contain (the author S.A. drew with the Biorender program), **b:** IGF-1R staining intensity graph; C: control, PS: pilonidal sinus

RESULTS

When the general tissue structure is examined with Masson's trichrome staining, the healthy epidermis (Fig.2/a and b) and the pilonidal sinus epidermal area (Fig.2/c) are compared, and a thin epidermis was observed in the PS. With Masson's trichrome staining, the medulla of the healthy hair structure was stained yellow, the sheath structures red, and the collagen fibers blue (Fig.2/a,b). In the PS, blood vessels in the connective tissue (Fig.2/c,d) and muscle/keratin fibrils stained light red (Fig.2/c,d), and collagen fibers stained light blue (Fig.2/c,d) were disorganized and thin in the pilonidal sinus, and draining material in the was located on the epidermal surface (Fig.2/c). Hair sheath structures were also scattered (Fig.2/d) in the PS. Hair follicles and cysts were embedded in the connective tissue in the PS tissues (Fig.2/d).

Immunohistochemically, IGF-1R staining was intense in melanized keratinocytes (Fig.3/a), cells located in the stratum basale in healthy tissues. Melanocytes were negatively stained (Fig.3/a). Staining was weak in gland structures (Fig.3/b). The staining of the basal line keratinocytes in the pilonidal sinus was significantly weakened and was negative in other structures and melanocytes (Fig.3/c). While bulbar melanocytes of hair follicles expressed IGF-1R in the healthy follicle (Fig.3/b), staining intensity was decreased in pilonidal sinus follicles (Fig.3/d). IGF-1R tissue staining intensity of pilonidal sinus cases was significantly reduced when compared to healthy skin ($p<0.05$) (Fig.1/b). The staining power of the markers of the examined tissues is shown in (Figure 4).

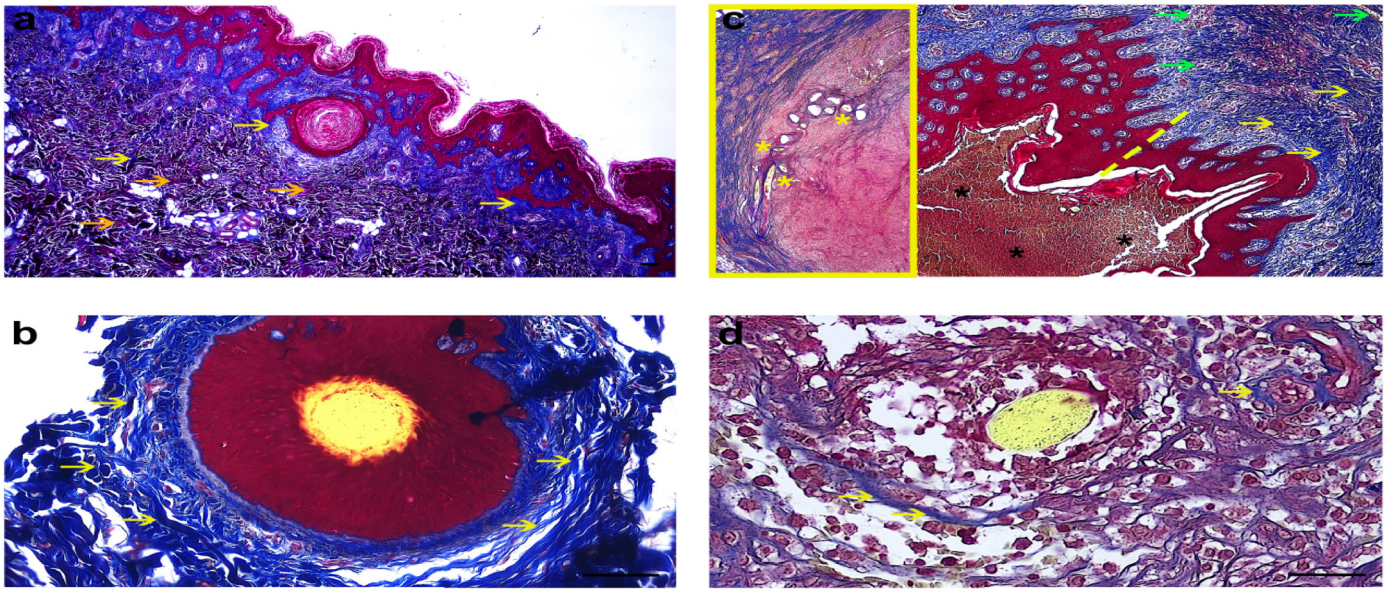

Figure 2. Masson's trichrome staining (a-d): over the yellow line; healthy skin, below the yellow line; epidermal area of pilonidal sinus, green arrows; blood vessel, orange arrows; muscle/keratin fibrils, yellow arrows; collagen fibers, black asterisks; inflammatory draining material, yellow asterisks; hair follicle and cysts, N: negative control, magnification; 5x,20x, scale bar; 50uu

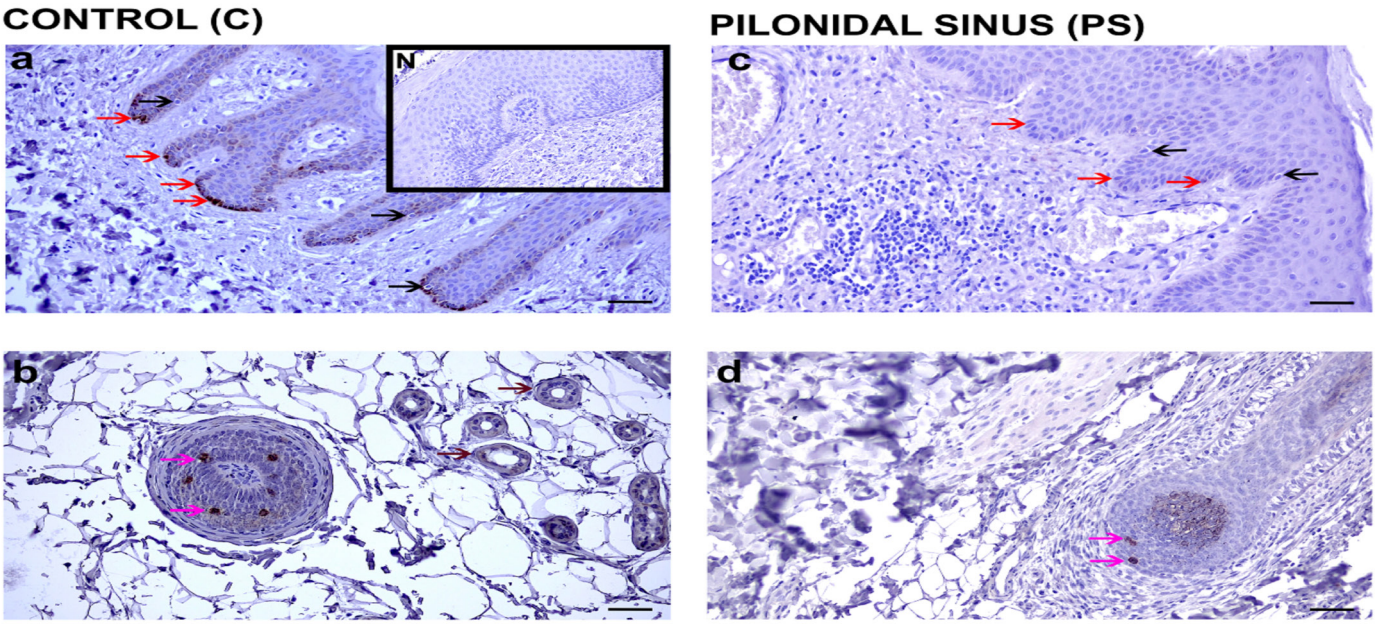

Figure 3. Immunohistochemical staining of IGF-1R (a-d): red arrows; melanized keratinocytes, black arrows; epidermal melanocytes, pink arrows; bulbar melanocytes, brown arrows; gland structures, N: negative control, magnification; 20x, scale bar; 50uu

H-SCORE		Control	PS
IGF-1R	Epithelium	(++)	(-)/(+)
	Keratinocytes	(+++)	(-)/(+)
	Melanocytes	(-)/(+)	(-)/(+)
	Muscles	(+)	(-)/(+)
	Glands	(+)	(-)/(+)
	Stroma	(++)	(+)
Masson's Trichrome Staining	Keratin	(+++)	(+)
	Collagen	(+++)	(++)
	Hair follicle	(+++)	(++)

Figure 4. Immunohistochemical staining power of markers (H-Score): non-staining; -/+, weak; +, median; ++, strong; +++

DISCUSSION

Pilonidal name; comes from the Latin pilus, meaning "hair," and nidus meaning "nest". The term "pilonidal disease" was coined by Hodges in 1880. It was defined by Mayo in 1833 and Anderson in 1847. During the wars, many US soldiers were diagnosed with the pilonidal disease, which has long been associated with driving, also known as "Jeep disease" (1). It was also named a sacrococcygeal cyst, often considering the congenital etiology of the disease (9).

In addressing the pathogenesis of pilonidal sinus disease, Karydakis attributed the hair regrowth process to three main factors: invasive, loose hair growth, a repulsive force that causes hair to settle, and the skin area around the birth cleft being too weak to prevent hair penetration. The sinus begins from a small midline opening lined by stratified squamous epithelium, and it is characterized by abscesses containing a sinus, hair, a cystic cavity lined with epithelial tissue, and a blunt-ended channel lined with granulation tissue (10). Loose hairs entering the subcutaneous tissues at the gluteal cleft are believed to cause foreign body response (11). Neutrophils and leukocytes are the structures found around abscesses in plasma cells and sometimes macrophages (10).

Although it is frequently seen in the sacrococcygeal region, it can sometimes be seen in the armpit, groin, between the fingers, navel, nose, breast area, suprapubic region, clitoris, foreskin, penis, occiput, and feet. In the sinus, the force from rubbing the skin at the base of the spine causes the hairs to sink below the surface. The hair forms small cavities or pits, enlarged hair follicles that become sinuses. Bacteria and debris enter this sterile area, causing local inflammation and the formation of pus-filled abscesses. In the chronic condition, the sinus becomes an open cavity that constantly drains a small amount of fluid (12).

Although there are many surgical techniques for treatment, no single method provides therapeutic success (3). It is widely believed that the ideal scenario in treating pilonidal sinus should be a technique with minimal excision, a low recurrence rate, a short hospital stay, a rapid return to normal life, and minimal scarring with minimal work loss (13). Moreover, It stands out in studies that indicate that extensive surgical procedures such as Z-plasty, rotated flap, or wide excision are unnecessary. There are studies advocating the view that marsupialization, which is a simple and accurate technique, gives excellent results with minimal recurrence (9).

After infection, the hair may penetrate the sinus wall or remain outside the sinus (14). Stelzner used light microscopy to indicate that hairs from pilonidal pits have a hook structure and suggested that hair migration is unidirectional. Dahl et al. confirmed the hook morphology and showed that the proposed sharp tips contribute to hair puncturing the skin, meaning hook formation prevents retraction. Gosselink et al. On the other hand, he states that the direction of the hair scales probably encourages the hair to be driven deeper into the tissue (15-17).

The hair, which is one of the skin appendages, and the hair follicle to which it is attached develop from hair follicle stem cells, which undergo growth, regression, and rest periods under the influence of external and internal factors and constitute one of the most typical examples of the stem cell niche. Hair growth cells surround the dermal papilla, and the crosstalk between mesenchymal cells and epithelial cells begins during embryogenesis when hairs first appear. After the first hair follicle is formed, the lifelong cycle of construction and destruction begins (5). Although there is information in the literature that pilonidal sinus formation is associated with structural weakness and skin inflammation, there is not enough data on the role of IGF-1R. For this reason, studies that will contribute to clarifying its histopathology will contribute to discovering unknown aspects. Our research observed that the connective tissue structure in which the hair follicle was located weakened, and the epidermis structure was thinned in the sinus region. Our findings support the hypothetical mechanism considering that proinflammatory cells are involved in this area.

The cells found in the stratum basale, located at the base of the epidermis layers of the skin, are cuboidal and mitotically active stem cells that continuously produce keratinocytes. Keratinocytes are the predominant cell type of the epidermis and originate from the basal layer, produce keratin, and are responsible for forming the epidermal water barrier by making and secreting lipids. Keratinocytes also have roles such as vitamin D synthesis and calcium absorption via UVB light activation. This layer also contains melanocytes. Melanocytes are derived

from neural crest cells. They are located between the cells of the stratum basale and produce melanin, which is associated with skin pigmentation. Long processes transfer melanin granules from melanocytes into the basal keratinocyte cytoplasm. Melanin is transferred to neighboring keratinocytes by "pigment donation" (18).

One of the vital growth factors found in the skin layers is insulin-like growth factor (IGF) (19). The IGF system contains three ligands (IGF-1, IGF-2, and insulin) belonging to a phylogenetically ancient peptide family involved in mammalian growth, development, metabolism, and cellular processes such as proliferation, survival, cell migration, and differentiation (20). IGF-1 is the primary regulator of longitudinal growth. IGF-2 is expressed in many tissues to regulate human pre-and and postnatal development (21). Deviations in the system of IGF-1, which has a high structural similarity to insulin, can be associated with various pathological conditions, including cancer. Insulin and its synthetic analogs are known to have IGF-IR binding affinity and mitogenic potential (22). IGF-1R has also been shown to interact with estrogen receptor signaling and cell-cell adhesion complexes (23). In BALB/c-3T3 fibroblasts, IGF-1 is required for cell cycle progression from G1 to S (DNA synthesis) phase, and its stimulatory role in cell proliferation is well established (24). It has been reported that epidermal basal keratinocytes are IGF-1 negative but IGF-IR positive. IGF-1 is thought to be an autocrine regulator of epidermal differentiation. The distribution of IGF-1R in the hair follicle indicates that they may be a morphogen rather than a mitogen in these regions because the IGF-IR of proliferating cells, not differentiated cells, is negative. Also, the expression of IGF-1R by the dermal papilla appears to be turned off during the transition from anagen to catagen. This implies a regulatory role for IGF-1 during the hair cycle (25). IGF-1 mRNA is expressed in the stratum granulosum of the epidermis and by dermal fibroblasts.

In human skin, epidermal keratinocytes do not express IGF-1, IGF-1R in keratinocytes is activated by IGF-1 secreted from dermal fibroblasts. Expression of IGF-1 is silenced in aged fibroblasts in vitro, and IGF-1 may be an essential component in the development of aging-associated non-melanoma skin cancer. Decreased expression of IGF-1 in aging skin is associated with an inappropriate UVB response in geriatric cases (26). In addition, mice's skin and epidermis layers carrying the gene mutations encoding IGF-1R are much thinner than the wild type. IGF-1/IGF-1R signaling plays a critical role during development. IGF-1 knockout mice die soon after birth, and these animals' skin barrier function and hair formation are impaired. In addition to a thinner skin formation, skin abnormalities are observed, including an impaired epidermis structure (27). Epidermal thickening develops in patients with acromegaly. It is thought that this situation may be related to promoting keratinocyte proliferation due to the increased secretion of IGF-1 mediated by growth hormone (28).

In studies of different systems, the hypothesis that insulin/IGF-1 signaling is involved in chronic inflammatory processes was demonstrated by Partridge et al. in insulin receptor substrate-1 deficient mice fully protected from age-related ulcerative dermatitis (29). Studies also strongly support the concept that IGF-1R activation in monocytes/macrophages controls the balance between proinflammatory and non-inflammatory macrophage populations during the development of skin inflammation (30).

When our study findings are evaluated together with the literature information mentioned above, It shows that IGF-1R is intensely expressed in healthy tissue, especially in keratinocytes. Keratinocytes have been associated with the modulation of UVB and skin aging in studies related to cancer. It appears to be important in maintaining a healthy skin structure. Moreover, IGF-1R is essential in keeping skin thickness, and the downregulation of its expression results in thin skin texture. We can say that we support Karydakisin's statement that a vulnerable skin structure and an impaired hair organization hypothetically play a role in the hair-settling process in pilonidal sinus formation. Because in our study, it was observed that IGF-1R, which is predominantly expressed in keratinocytes, is depressed in the pilonidal sinus, and maintaining a healthy skin structure may be interrupted in this case. Again, it can be said that this situation may support the formation of weak hair structure by disrupting the growth, production, and destruction diagram of the hair. On the other hand, this deterioration in growth factors is thought to be closely related to the worsening of epithelial organization and sinus formation. It has also been suggested that the IGF-1R receptor, which plays a role in the inflammatory process, may support an impaired anti-inflammatory response in these patients. We believe that a clear decision on whether IGF-1R replacement applications will improve the process in these patients can be made with more studies on the effects of IGF-1R.

CONCLUSION

In summary, when all findings and literature information are combined, it is thought that IGF-1R depression may be involved in the formation of the sacrococcygeal pilonidal sinus concerning the deterioration of the general organization of basal keratinocytes and the skin. However, more studies are needed on whether IGF-1R replacements can contribute to treatment.

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