Microarray-based exploration of molecules associated with keloid pruritus

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Abstract
Keloids are frequently accompanied with pruritus. Because traumatic stimuli such as scratching promotes keloid enlargement, alleviating pruritus is an important aspect of keloid management. To investigate the mechanism of keloid pruritus, we conducted a microarray-based comparison of keloid and nonkeloid skin. Relative expression levels of pruritus-associated ligands and receptors were determined, followed by construction of a heat map focused on serotonergic signaling, and finally pathway analysis. Results demonstrate relative up-regulation of various transcripts within keloid lesions, including those encoding a histamine receptor (H1R), a serotonin receptor (5-HT-2A), and an endothelin receptor (ET-A). Serotonergic signaling may be involved in keloid pruritus.

Keywords: Keloid, Itch, Serotonin, Endothelin, Histamine, Microarray

Keloids represent a unique hypertrophic connective tissue disorder that can occur following certain types of skin injury, including mechanical trauma, burns, folliculitis, and Bacille-Calmette-Guérin inoculation. After a variable period, keloid scarring spontaneously arises at the site of injury, taking on various characteristic appearances, including flat elevations with or without radial peripheral infiltration. Keloids represent tumors characterized by fibrosis, cartilage-like hardness, clear borders, and good mobility, and can appear anywhere on the body. Because keloids enlarge in response to external stimuli, they tend to be large, especially in areas prone to friction.

The major challenges of keloid management are tumorous growth and subjective symptoms. In addition to cosmetic complaints, patients frequently experience pain and pruritus (more pronounced in newer keloids)¹,², likely attributable to pruritogens produced by both the keloid parenchyma and infiltrating inflammatory cells. Lee et al.²¹ found that 86% of keloid patients experience pruritus, while 46% experience pain. Moreover, keloids demonstrate hypersensitivity to temperature differentials and painful stimuli². Because scratching increases keloid size, amelioration of pruritus and/or pain are important dimensions of keloid management. Elucidation of keloid molecular pathogenic mechanisms will facilitate improved prophylaxis and therapy. To date, only expression of TPH1, Cx43, and TRPV1 (involved in modulating cutaneous sensation) is known to be increased in keloid lesions³. Therefore, the present study employs a more comprehensive approach to elucidate the etiology of keloid pain and pruritus: comparing gene expression profiles of lesional and nonlesional skin from keloid patients.

Methods
Five surgical tissue samples (each consisting of a keloid lesion and surrounding normal skin) were obtained from 5 Japanese patients at the time of keloid removal. Samples were snap-frozen in liquid nitrogen until use. Experimental protocols were approved by the ethics committee of Nagasaki University Hospital (approval number 20170402), the study proceeded in accordance with the principles set forth in the Declaration of Helsinki, and informed consent was obtained from all study participants. Total RNA was separately extracted from lesion and normal tissues for each patient using an RNeasy Mini Kit (Qiagen, Valencia, CA), according to the manufacturer's instructions. Each RNA sample was assessed using Agilent Sure Print G3 Human Gene Expression 8x60K Microarray technology (Agilent Technologies, Palo Alto, CA). Expression profiles were analyzed using GeneSpring 12 (Agilent Technologies), followed by further data analysis using the R Project for Statistical computing (http://www.bioconductor.org/). Briefly, a modified paired t-test provided by the R Bioconductor package limma (L) was applied to identify significant between-group mRNA fold-change differences. Differences were considered significant at $P < 0.05$ and $|\log FC| > 1$. To investigate the potential role of serotonin in keloidal pruritus, a serotonergic signaling-focused heat map was constructed. Biological pathway enrichment analysis was performed using DAVID 6.8 (https://david.ncifcrf.gov/)³.© 2021 The Authors. Published by Wolters Kluwer Health, Inc. on behalf of the International Forum for the Study of Itch. This is an open access article distributed under the Creative Commons Attribution License 4.0 (CCBY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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Results

Demographic and clinicopathologic patient (N = 5) information is detailed in Table 1. Transcript expression ratios of known pruritogens and their receptors demonstrate that certain transcripts levels are higher in lesion samples in over 3 of 5 patients:

| Patient Information | Keloid Sex | Age (y) | Biopsy Site | Disease Duration (y) |
|---------------------|------------|---------|-------------|---------------------|
| 1                   | Female     | 33      | Abdomen    | 6                   |
| 2                   | Male       | 63      | Abdomen    | 10                  |
| 3                   | Male       | 50      | Abdomen    | 10                  |
| 4                   | Male       | 78      | Abdomen    | 1                   |
| 5                   | Male       | 48      | Chest      | 22                  |

**Table 1.** Patient demographic and clinicopathologic information.

Transcript expression ratios of pruritogens and their receptors in keloid tissue. A list of known pruritogens and their receptors was extracted from results of the microarray analysis, indicating up-regulated transcription in keloid lesions of genes encoding receptors for histamine (H1R), serotonin (5-HT-2A), and endothelin (ET-A). Numbers represent log2N. Color key: pink up-regulated genes (log2N > 1.0), orange moderately up-regulated genes (log2N > 0), green moderately down-regulated genes (log2N < -0.5), blue down-regulated genes (log2N < -1.0).

Figure 1. Transcript expression ratios of pruritogens and their receptors in keloid tissue. Asai et al. Itch (2021) 6:e54

Discussion

As mentioned, keloid scarring results due to excess extracellular matrix component deposition during abnormal wound healing.
Keloid-associated physical symptoms such as pain and pruritus greatly decrease patient quality of life[4]. While various genetic and systemic factors are associated with the increased inflammation and proliferation, and decreased remodeling, implicated in keloid formation, molecular pathogenesis remains incompletely understood. Current treatments focus primarily on decreasing inflammation, and while therapeutic interventions targeting specific cytokines or other molecules have been proposed, developing more effective prophylactic and therapeutic strategies for keloid development and associated pruritus requires a better understanding of the molecular mechanisms underlying keloidogenesis. Therefore, the present study investigated global gene transcription in both keloid and nonkeloid skin, and subsequently focused on pruritogen receptors demonstrating up-regulated expression in lesions.

Results indicate that lesions exhibit increased transcription of genes encoding H1R, ET-A, and 5-HT-2A. While involvement of H1R and ET-A in fibrosis during keloidogenesis has been well described[5–8], to the best of our knowledge this is the first report of 5-HT-2A involvement. Histamine, a prototypical pruritogen, significantly promotes proliferation of human keloid-derived fibroblasts in vitro. This phenotypic difference in the degree of growth stimulation in response to histamine may be due to epigenetic alterations[6]. Fibroblasts are more abundant in keloid tissue than in tissues exhibiting normal collagen production[9]. Histamine induces fibroblast collagen production via the H1 receptor[7]. Tranilast, an antiallergic drug that inhibits the release of substances such as histamine and prostaglandins from mast cells, can suppress collagen synthesis by suppressing the release of TGF-β1 from keloid-derived fibroblasts in vitro[9,10]. While antihistamines can be expected to inhibit keloid formation, few data demonstrating the clinical efficacy of antihistamines for keloid-associated pruritus are available; this is a topic for further study.

Endothelin-1 is a peptide involved in pruritogenesis via ligation of the ET-A receptor[11,12]. It has been reported to promote fibrosis within scar tissue (including keloids) by inducing myofibroblasts[8], and is implicated in the pathogenesis of keloidogenesis and associated pruritogenesis. Therefore, the endothelin-1/ET-A receptor axis has been proposed as a therapeutic target in these contexts.

Serotonin, also known as 5-hydroxytryptamine (5-HT), signals via 5-HT receptors. Diverse 5-HT receptor subtypes trigger a variety of distinct cellular responses. Platelet-derived serotonin is known to be important for normal skin healing, and 5-HT is involved in promoting formation of scaffold tissue, angiogenesis, and inflammatory responses during restoration of normal tissue architecture. However, in chronic skin wounds, excessive 5-HT signaling promotes abnormal healing and may be involved in keloid formation. In particular, it has been proposed that 5-HT-2A activity may be involved in keloid pathogenesis[13], and a 5-HT-2A receptor antagonist attenuates spontaneous pruritus in a murine model of chronic dry skin[14]. The present study further indicates up-regulated transcription of the genes encoding VGCC, PKC, and KCN in keloid lesions. These molecules are known to modulate neuronal excitability and synaptic transmission.

The involvement of these genes in keloid pain remains unclear. As far as we know, no previous reports have shown a relationship between 5-HT-2A or ET-1 and keloid pain. ET-1-mediated or 5-HT-2A-mediated signals can result in both pain and analgesia, depending on cell type[15–18]. Given the dichotomous effects of ET-1 and 5-HT-2A on pain, further studies exploring other candidate genes related to keloid pain are necessary.

Limitations of our study include: a sample preparation process that could not predicate a direct link between candidate genes and keloid itch and the lack of a subjective assessment for itch intensity in study subjects. In conclusion, further studies are needed to support our results implicating serotonin signaling (especially via 5-HT-2A) in keloidogenesis and associated pruritus. These findings support further investigation into the impact of 5-HT-2A antagonists on keloid pathogenesis and existing keloids.

Presentation
The authors submitted the content in the World Congress of Itch (WCI) 2019 as a poster presentation.

Conflict of interest statement
The authors declare that they have no financial conflict of interest with regard to the content of this report.

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