influence the genotype–phenotype relationship (Covello *et al.*, 1998; Leachman *et al.*, 2005). No patients have been reported with K6b mutations and pure steatocystoma multiplex (McLean *et al.*, 2005), but our patient does have multiple flesh-colored papules which extrude greasy contents, consistent with steatocystomas.

Treatment for pachyonychia congenita is limited and fairly unsuccessful by current standards. The most debilitating feature as reported by patients is plantar pain, with the most promising treatments thus far consisting of activities to reduce friction and botulinum toxin to decrease sweating (Leachman *et al.*, 2005; Milstone *et al.*, 2005). Other features, such as hyperkeratosis of the nails, cysts, and mucous membrane lesions are managed by symptomatic treatment ranging from use of humectants and mechanical removal of keratin in thick nails to excision or incision and drainage of cysts. Fortunately, newer and better gene-based treatments are on the horizon. Exciting options being considered include triplex-forming oligonucleotides or zinc-finger nucleases to inactivate or induce correction of the mutant allele and most promising to date, the use of targeted small inhibitory RNAs to silence gene expression of the mutant allele (Lewin *et al.*, 2005; Hickerson *et al.*, 2006). Hopefully, with continued research into the genetics of pachyonychia congenita, the goal of gene-based therapy will become attainable.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

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***Vishakha M. Sharma*[1] *and Sarah L. Stein*[2]**

*[1]Department of Medicine, Section of Dermatology, University of Chicago, Chicago, Illinios, USA and [2]Department of Medicine and Pediatrics, Section of Dermatology, University of Chicago, Chicago, Illinios, USA*
*E-mail: Vishakha.sharma@uchospitals.edu*

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# P75 Plays a Key Role in the Induction of the Sprouting of Sensory Nerve Fibers in Inflamed Skin

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## TO THE EDITOR

In hyperkeratosis and acanthosis, peripheral branches of sensory nerves increase remarkably in number, particularly in the epidermis of inflamed skin (Mihm *et al.*, 1976; Pincelli *et al.*, 1990; Tobin *et al.*, 1992; Ostlere *et al.*, 1995). It has been supposed that nerve growth factor (NGF) secreted from keratinocytes in inflamed skin may induce the sprouting of the neurites of sensory fibers (Dou *et al.*, 2006) because of an increase in both NGF expression in the keratinocytes (Kinkelin *et al.*, 2000; Takano *et al.*, 2005; Tanaka and Matsuda, 2005) and cutaneous innervation in the epidermis in the inflamed skin (Horiuchi *et al.*, 2005). The NGF effect may occur via low-affinity receptors (p75) and high-affinity receptors (TrkA), although direct evidence for this is lacking. This study clearly demonstrates, by using p75 knockout mice, that sprouting of sensory fibers in the epidermis and hyperkeratosis and acanthosis of the inflamed skin are induced by an NGF–p75 pathway.

*Abbreviations: NGF, nerve growth factor; PC, picryl chloride (2,4,6-trinitrochlorobenzene)*

Five female mice with a C57BL/6j background and *p75* gene mutation (Lee *et al.*, 1992) (Jackson Laboratory, Maine) were used in this study. The anterior abdominal skin was painted with 100 $\mu$l of a 5% picryl chloride (PC) solution (Tokyo Kasei Kogyo, Tokyo, Japan) for the initial sensitization. The cutaneous reaction in the sensitized C57BL/6j and p75 knockout mice was evoked by repeated paintings of both ears with 15 $\mu$l of 1% PC solution. Controls received repeated paintings of the ears with 15 $\mu$l of solvent without the PC. The PC challenge was repeated once a week for 4 weeks, followed by three times per week, 10 times in total, from 7 days after the initial sensitization. Histochemistry for substance P, protein gene product 9.5, NGF, and p75 was then performed. The specificity of the immunoreaction for substance P and NGF was confirmed by the absorption test. Specificity for p75 immunoreaction was checked in p75 knockout mice (Figures 1g, h and 2h, i). As a preliminary experiment, PC treatment was found to cause inflammation of the skin of two male NC/Nga Tnd Cr1j mice. All animal experiments were carried out in accordance with a protocol approved by the institutional Animal Care and Use Committee of Osaka University.

PC treatment on the C57BL/6 mouse skin resulted in a remarkable hyperkeratosis and acanthosis (Figure 1). Figure 1a–d shows the changes of the localization of substance P fibers and protein gene product 9.5 fibers in the epidermis of C57BL/6 mice after PC treatment, which caused sprouting of sensory fibers in the epidermis of the inflamed skin (Figure 1b and d). Figure 1e–h shows the alteration of NGF and p75-like immunoreactivity in the PC treatment C57BL/6 mice. NGF was weakly expressed in the keratinocytes of C57BL/6 mice without the PC treatment (Figure 1e). In the PC-treatment mice, immunoreactivity in the keratinocytes increased in number in the epidermis (Figure 1f). No NGF-positive fibers were seen in the normal or inflamed skin. The alteration of p75 expression in the keratinocytes was similar to that found with NGF (Figure 1g and h). The most remarkable change found in the expression of p75 in the inflamed skin was the presence of a number of p75-positive fibers in the epidermis (Figure 1h), where no p75-labeled fibers were seen in the normal epidermis (Figure 1g inset).

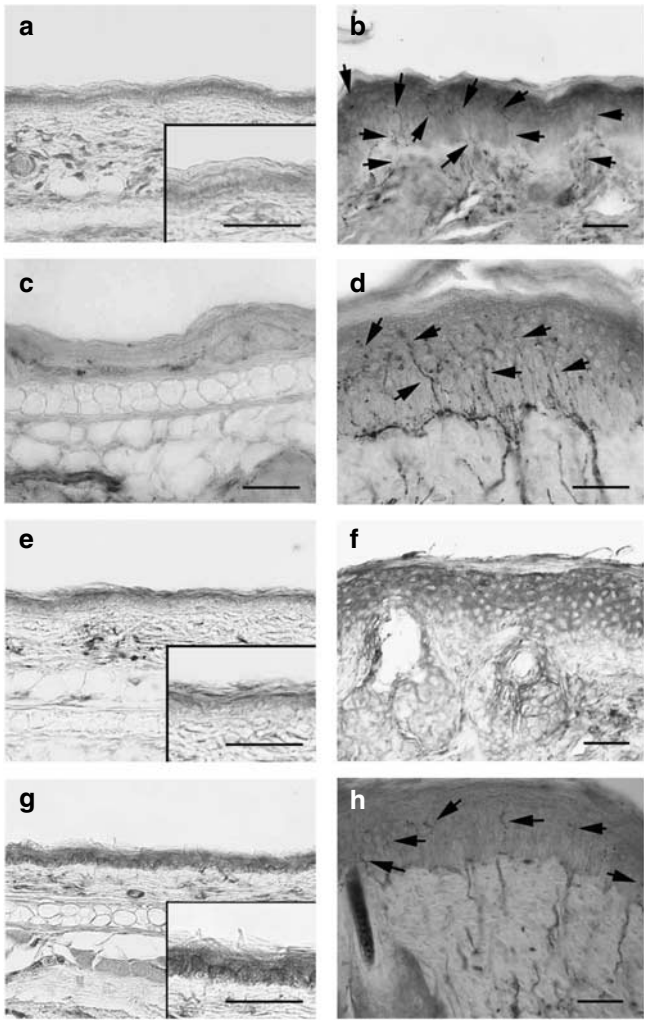


**Figure 1. The histological analysis of the epidermis in C57BL/6 mice following PC treatment.** (**a** and **b**) Changes in the immunoreactivity for substance P, (**c** and **d**) protein gene product 9.5, (**e** and **f**) NGF, and (**g** and **h**) p75 in the epidermis (auricle) of C57BL/6 mice (**a**, **c**, **e**, and **g**) without PC treatment and (**b**, **d**, **f**, and **h**) with PC treatment are shown. (**a**–**d**) The sprouting of sensory nerve fibers containing substance P and protein gene product 9.5 in the epidermis (auricle). In the control mice, as shown in inset of (**a** and **c**), a few substance P and protein gene product 9.5 fibers were seen in the epidermis. (**b** and **d**) In the PC-treated mice, substance P and protein gene product 9.5-positive fibers (arrows) were seen in the epidermis. (**e** and **g**) In the control mice, keratinocytes were weakly immunoreactive for NGF and p75 in the epidermis. (**f** and **h**) In the PC-treated mice, hyperkeratosis and acantosis were identified and strong immunoreactivity for NGF and p75 was observed in the epidermis. Only a few P75-immunostained cells were seen in the basal layer of the epidermis (inset of **g**). As for the p75 expression, in the control mice, few P75 fibers were seen in epidermis, whereas many P75-positive nerve fibers (arrows) were seen in the epidermis of the (**h**) PC-treated mice. Bar = 50 $\mu$m.

In contrast to C57BL/6 mice, in the p75 knockout mice, hyperkeratosis and acanthosis were inhibited remarkably after PC treatment (Figure 2a–c). In addition, no sprouting of substance P fibers was identified in the epidermis in p75 knockout mice, both with and without PC treatment (Figure 2d and e). PC treatment of p75 knockout mice failed to increase the number of NGF-expressing keratinocytes compared with the PC-treated wild-type mice (Figure 2f and g). In the epidermis of p75 knock-