suggests that further clarification of the role of PAX3 expression in non-malignant differentiated melanocytes is now required. Extensive PAX3 expression in benign and malignant tissues of the melanocyte lineage, and absence of expression in other types of skin cancer, nevertheless suggests that PAX3 could be used as an immunohistochemical marker to differentially diagnose melanoma.

Ethics approval for this study was obtained from the New Zealand Multi-Regional Ethics Committee.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Cathepsin S Elicits Itch and Signals via Protease-Activated Receptors

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TO THE EDITOR

Cathepsin S is a cysteine protease linked to inflammatory processes, including atherosclerosis and asthma. The possibility that this or other cysteine proteases might evoke itch or be part of a classical ligand-receptor signaling cascade has not been considered previously. We show here that human cathepsin S evokes itch and activates human protease-activated receptors (PARs) 2 and 4.

The sensation of itch is mediated by two distinct non-overlapping populations of cutaneous nerve fibers that evoke comparable degrees of itch (Johanek *et al.*, 2008; Namer *et al.*, 2008). One set of fibers, the mechano-insensitive population, is more responsive to histamine than to cowhage. The other set is mechanosensitive and is more responsive to cowhage than to histamine (Johanek *et al.*, 2008; Namer *et al.*, 2008). Histamine is a classical mediator of itch and is associated with a wheal and flare. As most clinical itches do not have a wheal or flare and do not respond to antihistamines, histamine is not thought to contribute to most itches (Ikoma *et al.*, 2006). Cowhage refers to a tropical legume or, in this case, the loose hairs that cover the pods of *Mucuna pruriens* and evoke itch. The active component of cowhage is mucunain, a cysteine protease that serves as a ligand for PARs 2 and 4 (Reddy *et al.*, 2008). The identification of an endogenous mediator with properties similar to cowhage could lead to insights into non-histamine-mediated itch. We focused on human cathepsin S because it shares active site sequence homology with mucunain and is selectively up-regulated in human keratinocytes upon stimulation with interferon-gamma, consistent with a possible pruritic role in inflammatory skin disease (Schwarz *et al.*, 2002).

Abbreviation: PAR, protease-activated receptor

There are 15 human cathepsins, including 11 cysteine, 2 aspartic, and 2 serine proteases. Cathepsins were traditionally considered lysosomal proteases. It is now recognized that the broad expression and range of pH dependence of some cathepsins reveal that they have many functional roles, including tissue remodeling, metastasis and inflammation. Examples of cysteine cathepsin activities include cleavage of collagen by cathepsin L to generate endostatin (Felbor *et al.* 2000), an endogenous inhibitor of angiogenesis, and cleavage of the invariant chain in antigen-presenting cells by cathepsin S (Nakagawa *et al.*, 1999) as part of the inflammatory cascade.

There are four PARs and they are members of the G-protein-coupled receptor family. Their identified endogenous activators are all serine proteases. These proteases trigger the activation of PARs by unmasking 'tethered ligand' sequences near the N-termini of the receptors. Certain kallikrein-related peptidases and mast

cell tryptase, which are serine proteases, can activate PAR2 *in vitro* (Oikonomopoulou *et al.*, 2006, Stefansson *et al.*, 2008), but they have not been demonstrated to be endogenous pruritic agents. Serine proteases and PAR2 have also been linked to barrier function (Hachem *et al.*, 2006). The presence of PAR2 on free nerve endings in the skin, keratinocytes and dorsal root ganglia link this receptor to itch and pain (Steinhoff *et al.*, 2000, 2003; Shpacovitch *et al.*, 2008). Data on PAR4 are more limited, revealing that it is expressed in rat dorsal root ganglia and its activation, at least in this species, appears to be anti-nociceptive (Asfaha *et al.*, 2007). A PAR4 hexapeptide agonist has recently been reported to cause scratching in mice (Tsujii *et al.*, 2008).

Cathepsin S and mucunain are endogenous and exogenous cysteine proteases, evoke similar itch and nociceptive sensations, and serve to activate PARs 2 and 4 (Figures 1 and 2; Reddy *et al.*, 2008). The relative contribution of PAR2 versus PAR4 activation in cathepsin S-induced sensations is not addressed by these data. Cathepsin S has been implicated in neuropathic pain, manifested by increased gene expression in rat dorsal root ganglia following peripheral nerve injury. Such pain was lessened by a cathepsin S inhibitor (Barclay *et al.*, 2007). These observations suggest that cathepsin S may have an excitatory effect on neurons. As keratinocytes have been demonstrated to express cathepsin S (Schwarz *et al.*, 2002), a role for this protease in keratinocyte-neuronal communication may be expected. Cathepsin S may contribute to the pruritus of inflammatory skin conditions, including atopic dermatitis and psoriasis, and could have a role in barrier function. Cathepsin S and other endogenous or exogenous cysteine proteases may activate PARs as a part of additional inflammatory processes. For example, Der p1, a mite cysteine protease associated with asthma, activates PAR2 (Asokananthan *et al.*, 2002). Taken together, we suggest that exogenous and endogenous cysteine proteases interact with PARs as a part of signaling cascades in homeostasis and disease.

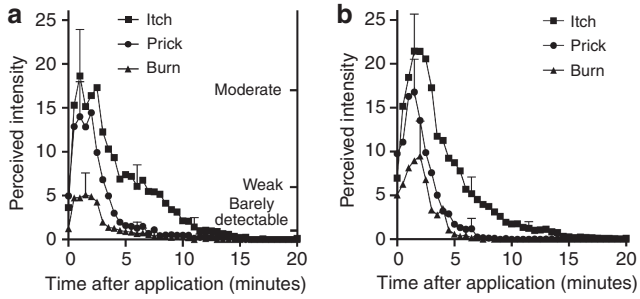

Figure 1. Recombinant human cathepsin S evokes itch. (**a**) Mean perceived intensity of itch, pricking/stinging, and burning sensations evoked by a single inactivated spicule reconstituted with cathepsin S. Briefly, spicules were inactivated by autoclaving and then soaked in a solution containing 4 mg ml^{-1} cathepsin S for 1 hour, washed, and dried. The spicules were applied to the skin, and nine, healthy, unmedicated volunteers (four female, five male, 18 years or older) rated the evoked sensations, according to similar methods described (Reddy *et al.*, 2008). The mean rating of each sensory quality, obtained every 30 seconds, is accompanied by an SE that is plotted every 5 minutes starting at the peak magnitude of the sensory quality. (**b**) Mean perceived intensity of sensations evoked by a single spicule of native cowhage (the same nine subjects).

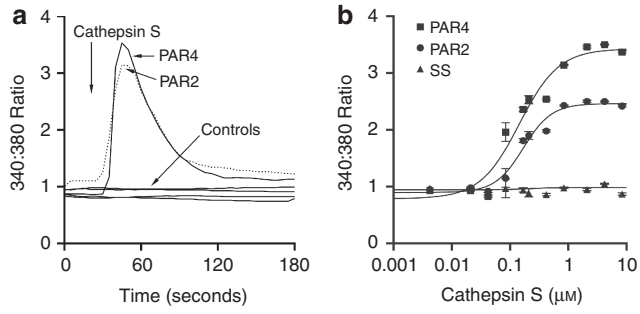

Figure 2. Recombinant human cathepsin S activates human protease-activated receptors (PARs). (**a**) Single-cell imaging of cathepsin S (2 μM) induced responses in HeLa cells transfected with either PAR 2 or PAR 4 as measured by ratiometric calcium imaging in cells loaded with fura-2 as in Reddy *et al.* (2008). The responses to PARs 2 and 4 were blocked by the protease inhibitor E64 (10 μM) as a control. Other controls included vector alone and PAR1. Cathepsin S was added as indicated. (**b**) Concentration–effect responses of cathepsin S in HeLa cells transiently transfected with PARs 2 and 4 as determined by ratiometric imaging of 20 cells per data point. Salmon sperm (SS) DNA as control. For both (**a**) and (**b**), the averages from at least three separate experiments were then combined ± SD (see Supplementary material online).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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IRF4 Expression without *IRF4* Rearrangement Is a General Feature of Primary Cutaneous Diffuse Large B-Cell Lymphoma, Leg Type

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TO THE EDITOR

The involvement of the interferon regulatory factor 4 gene (*IRF4*), also known as multiple myeloma antigen 1 (*MUM1*), in balanced rearrangement or translocation has been recently observed in a subset of cutaneous T-cell lymphomas (CTCLs), such as cutaneous anaplastic large-cell lymphoma (C-ALCL) and transformed mycosis fungoides (Feldman *et al.*, 2009; Pham-Ledard *et al.*, 2009). *IRF4* expression reaches a high level in differentiated plasma cells and is also detectable by immunostaining in some activated T cells and melanocytes, with the latter providing internal positive controls on skin sections (Falini *et al.*, 2000; Lu, 2008). Despite such a restricted immunostaining pattern, *IRF4* is an essential regulator at multiple steps of B-cell differentiation, such as pre-B-cell differentiation, germinal center formation, immunoglobulin class switch recombination, and terminal differentiation of B cells to plasma cells, as shown in *IRF4*-deficient mice (reviewed in Shaffer *et al.*, 2009). IRF4 is also essential for T-helper (Th) cell differentiation and is required for either Th2 or Th17 cell development (Brustle *et al.*, 2007; Zheng *et al.*, 2009). An oncogenic role of *IRF4* has first been supported by the identification of *IRF4* involvement in the t(6;14)(p25;q32) translocation in some cases of multiple myeloma (MM) (Iida *et al.*, 1997). In t(6;14)(p25;q32), IRF4 is juxtaposed with the immunoglobulin heavy-chain gene locus leading to *IRF4* deregulated expression (Iida *et al.*, 1997; Yoshida *et al.*, 1999; Shaffer *et al.*, 2008). Alternatively, *IRF4* rearrangements in peripheral T-cell lymphoma do not commonly involve either the *TCRB* or the *TCRA* gene locus, as shown in eight C-ALCL and two systemic T-cell lymphomas (Feldman *et al.*, 2009).

Among primary cutaneous B-cell lymphomas, primary cutaneous diffuse large B-cell lymphoma, leg type (PCLBCL, leg type) is an original entity with poor prognosis that was first reported in 1996 and that mostly affects the leg(s) in elderly but may also arise at other sites in approximately 10% of cases (Vermeer *et al.*, 1996; Willemze *et al.*, 2005; Meijer *et al.*, 2008). PCLBCL, leg type, differs from primary cutaneous follicle center lymphoma by the presence of confluent sheets of centroblasts and immunoblasts, many with a peculiar round cell morphology, which strongly express B-cell CLL/lymphoma 2 (BCL2), IRF4, and forkhead box P1 (FOXP1) (Willemze *et al.*, 2005; Meijer *et al.*, 2008). Interestingly, round cell morphology, strong BCL2

Abbreviations: FISH, fluorescence in situ *hybridization; IRF4, interferon regulatory factor 4; MM, multiple myeloma; MUM1, multiple myeloma antigen 1; PCLBCL, leg type, primary cutaneous diffuse large B-cell lymphoma, leg type*