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Identification of a group of proteins that are strongly up-regulated in total epidermal keratinocytes from psoriatic skin

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Analysis using two-dimensional (2D) gel electrophoresis of the [^{35}S]-methionine-labelled proteins synthesized by non-cultured total epidermal keratinocytes obtained from normal and psoriatic skin revealed 6 proteins that are strongly up-regulated (5 times or more) in psoriatic skin. These proteins are synthesized at albeit lower levels by keratinocytes from normal and normal-appearing (uninvolved) skin of psoriatic patients, and correspond to isoelectric focusing sample spot numbers 4311 (40.3 kDa), 4003 (12.4 kDa), 5008 (11.9 kDa), 3012 (11.6 kDa), 6016 (11.6 kDa) and 1015 (10.1 kDa) in the normal keratinocyte 2D gel protein database [Celis et al, (1990) Electrophoresis, in press]. These proteins are also detected in the labelling medium indicating that they are at least in part secreted. Given their striking regulatory behavior, these proteins may play a role in the pathogenesis of psoriasis.

Skin disease; Psoriasis; Protein sythesis; Marker; Two-dimensional gel protein database

1. INTRODUCTION

Psoriasis is a chronic skin disease of widespread occurrence characterized by epidermal hyperplasia (acanthosis) and inflammation with infiltrate of polymorphonuclear leukocytes, activated T cells, Langerhans cells and macrophages [1-4 and references therein]. At present, little is known about the molecular mechanisms underlying the pathogenesis of this disease, although increased local production of growth factor(s) or cytokine(s) has been implicated in the phenomena [2,3,5-8]. Recently, Grossman et al. [9] have presented direct evidence indicating that IL-6 may play a role in the epidermal hyperplasia observed in the psoriatic skin. In this article, we describe the detection of 6 secreted proteins that are produced, albeit at a reduced rate, by normal human keratinocytes and that are strongly up-regulated in psoriatic epidermis.

2. MATERIALS AND METHODS

2.1. *Patients and skin biopsies*

Patients with plaque psoriasis on the limb (fig.1) or trunk that were resistant to all forms of treatment were studied. Dermatome shaving was carried out under local anesthesia in the outpatient clinic [10]. Serial shaving were done to a depth at which bleeding occurred from the dermal communicating vessels. At that level, the cut surface had the uniform white colour of the reticular dermis, whereas in the more superficial layers there was patchy yellow staining, presumably confined to areas where the thick psoriatic parakeratotic epidermis remained [10]. Samples of normal skin tissue were obtained from healthy volunteers and from normal appearing (uninvolved) skin of psoriatic patients.

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Abbreviations: 2D, two dimensional; IEF, isoelectric focusing; SSP, sample spot number; IL-6, interleukin-6; DMEM, Dulbecco's modified Eagle's medium

2.2. *Preparation of total human epidermal keratinocytes*

Strips of skin (normal, uninvolved, or psoriatic, about 1 g) were washed 3 times in Hank's buffered saline and placed in 10 ml of 0.25% trypsin in Hank's saline (Gibco, 1:250) at 4°C for 15-17 h. Following incubation, the strips were washed 3 times in DMEM containing 10% fetal calf serum and the epidermis was detached from the dermis by using fine forceps [11,12]. The epidermal samples were then carefully washed 3 times in DMEM containing 10% sera and finally resuspended in 8 ml of the same solution. The samples were then shaken vigorously in a 10 ml plastic tube to detach basal and suprabasal cells. The suspension of epidermal cells was allowed to stand for a few minutes at room temperature and the upper 5 ml of suspension containing single cells and small aggregates were aspirated with a 10 ml pipette. Epidermal keratinocytes were labelled in suspension (non-cultured) with [^{35}S]-methionine immediately after preparation (see below).

2.3. *Labelling of non-cultured keratinocytes with [^{35}S]-methionine*

Total epidermal cells in suspension (0.3 ml) were pelleted by centrifugation (2000 × *g* for 3 min) and resuspended in 0.3 ml of laboratory-made DMEM (1 g/l, $NaHCO_3$) lacking methionine and containing 10% dialyzed fetal calf serum and 150 μCi of [^{35}S]-methionine (SJ 204, Amersham) [13]. After labelling for 14 h, the medium was removed with the aid of an elongated Pasteur pipette and the cells were resuspended in 200-300 μl of lysis solution [14]. The medium containing secreted proteins was centrifuged at 2000 × *g* for 5 min to eliminate contaminating whole cells, freezed dried and resuspended in 200 μl of lysis solution [14].

The procedures for running 2D gels and for computer-analyzed 2D gel electrophoresis have been previously described in detail [15].

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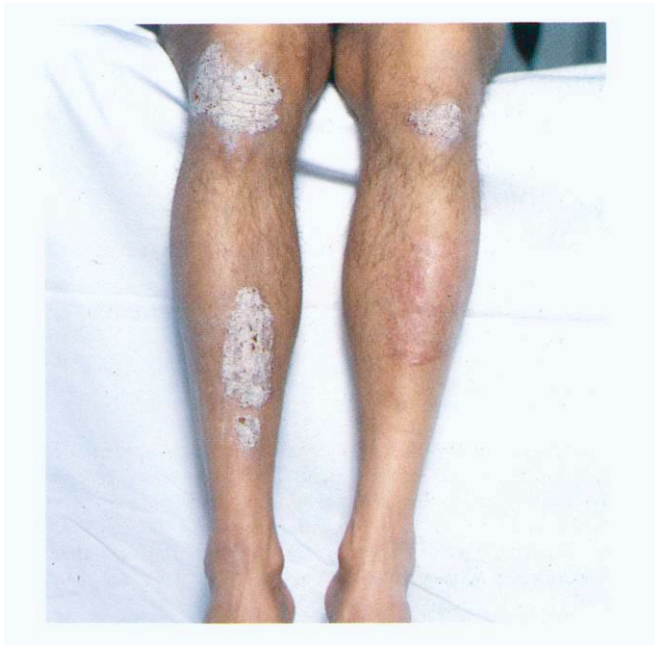

Fig.1. Plaque psoriasis on the leg of a 30-year-old man. Psoriasis plaque on the left leg has been removed by dermatome shaving.

3. RESULTS AND DISCUSSION

Fig.2 shows an IEF gel [^{35}S]-methionine-labelled cellular proteins from total human epidermal keratinocytes obtained from mammary epidermis of a normal individual. Very similar patterns have been observed in the case of keratinocytes obtained from leg epidermis. The position of some known proteins, including those identified in this work, are indicated for reference. The upper panel in fig.3 shows a synthetic image of the gel depicted in fig.2. 1140 IEF polypeptides ranging in molecular mass from 9.4 to 204 kDa have been recorded in the keratinocyte 2D gel protein database [16]. The lower panel in fig.3 shows some annotations entered under the category protein name. A detailed description of the 2D gel keratinocyte protein database will appear elsewhere [16].

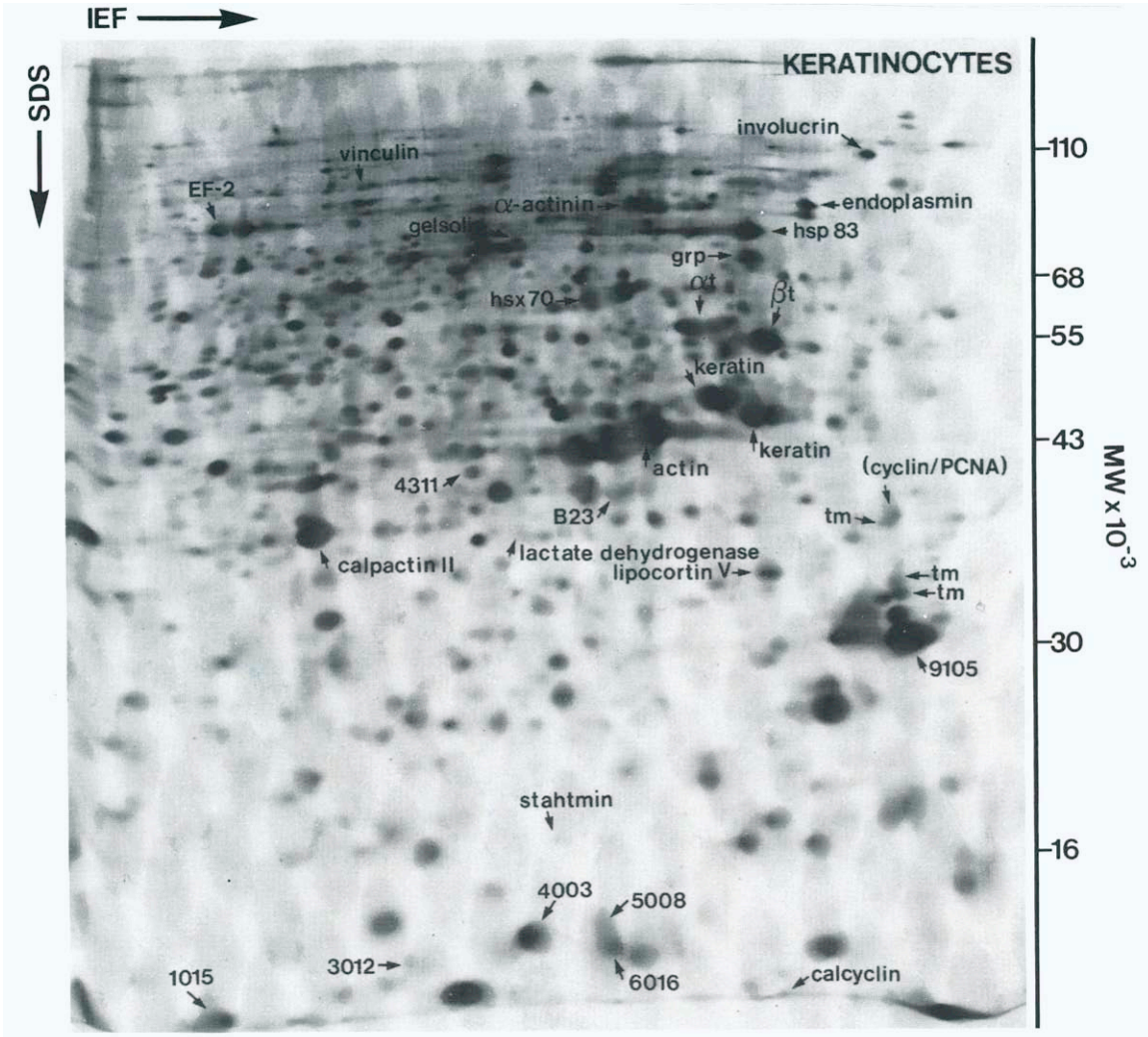

Fig.2. 2D gel (IEF) fluorograms of [^{35}S]-methionine-labelled polypeptides from total human epidermal keratinocytes obtained from normal mammary epidermis. A few known proteins are indicated for reference. grp, glucose regulated protein; EF-2, elongation factor 2; tm, tropomyosin (for further information see [16]).

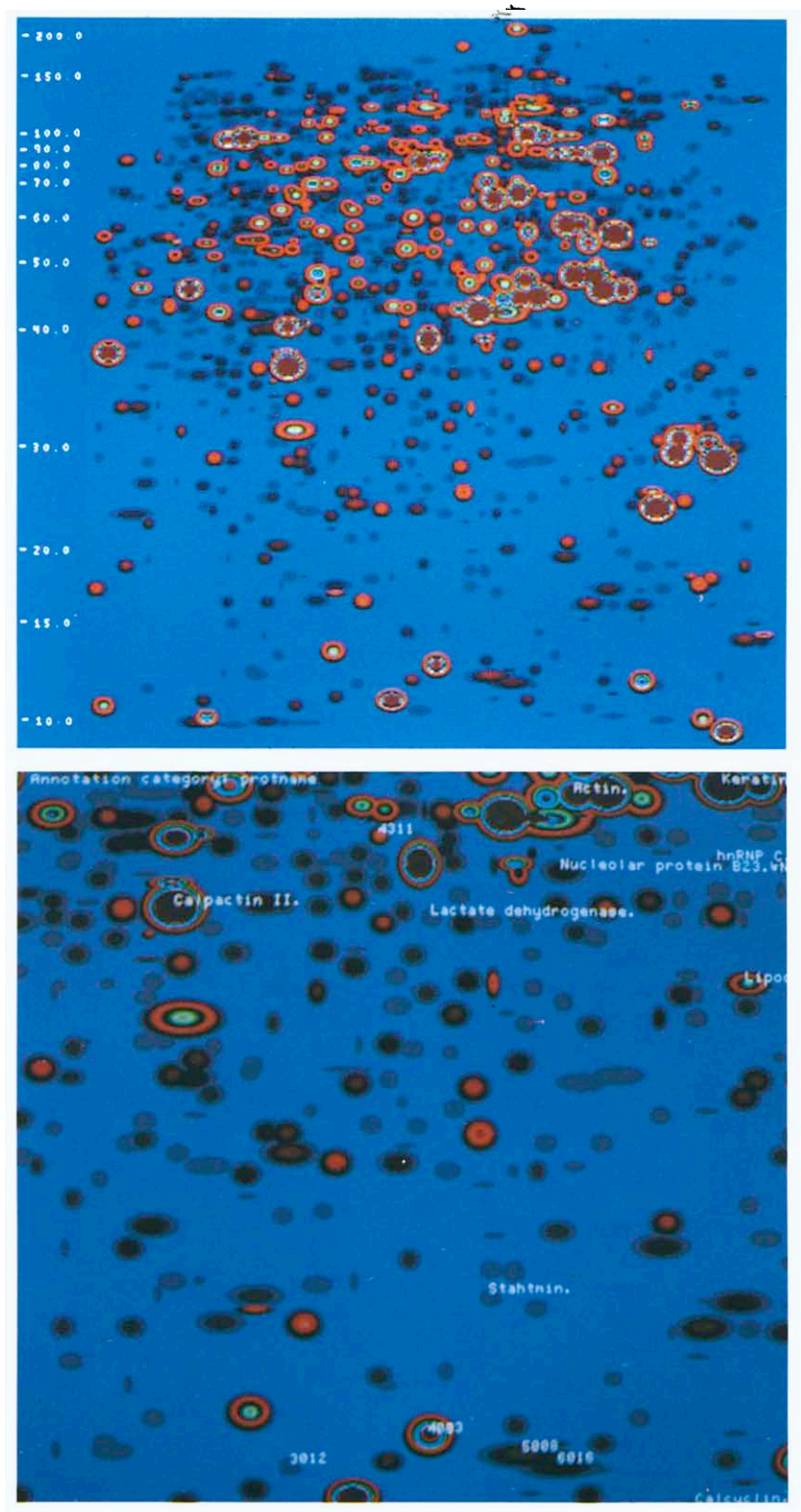

Fig.3. Synthetic images of IEF gels of [^{35}S]-methionine-labelled proteins from total epidermal keratinocytes from normal mammary epidermis. Upper panel, total proteins (same gel as that shown in fig.2). Lower panel, fraction of the image shown in the upper panel displaying information contained in the entry 'protein name'. For further details, see [16].

Fig.4A and B show fractions of IEF gels of [^{35}S]-methionine-labelled proteins from total keratinocytes obtained from normal appearing skin (uninvolved region about 1 cm outside the psoriasis plaque; fig.4A) and psoriatic plaques (fig.4B) from the same patient (see also fig.1). Immunofluorescence pictures of

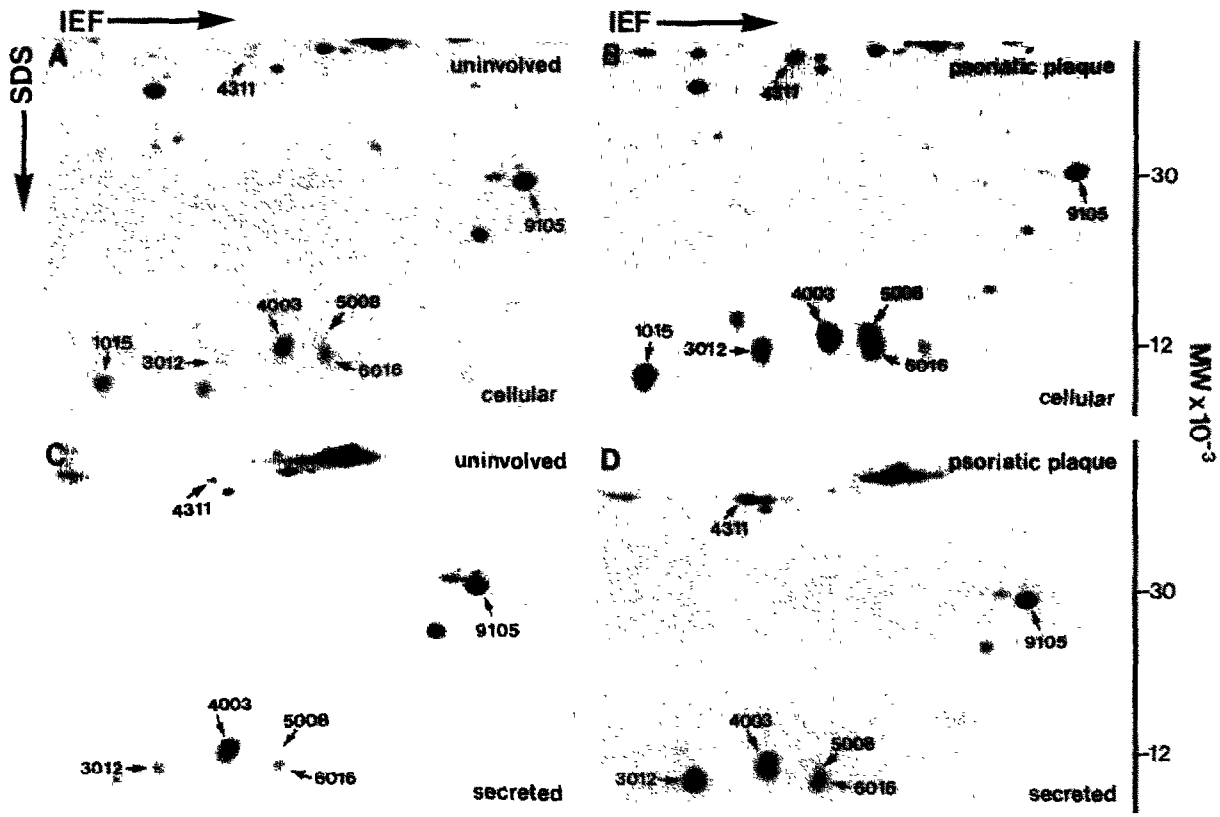

Fig.4. 2D gel (IEF) fluorograms of [^{35}S]-methionine-labelled proteins of total epidermal keratinocytes obtained from (A,C) normal appearing (uninvolved region about 1 cm outside the psoriasis plaque) and (B,D) psoriatic plaques from the same patient. A, B, cellular proteins. C, D, secreted proteins. Protein IEF 1015 did run out of the gel in C and D.

methanol-fixed cryostat sections from uninvolved and psoriatic plaques reacted with a monoclonal antibody specific for basal keratinocytes (mAB BG3C8) [11,12,17] are shown in fig.5A and B, respectively. Clearly, the basal cell antigen is expressed by many suprabasal cells in the psoriatic epidermis (Fig.5B, cf. Fig.5A) in line with the fact that these cells exhibit abnormal cell proliferation and differentiation. In short, the 2D gel analysis of many independent samples from psoriatic patients revealed 6 proteins that were highly up-regulated (5 times or more) in the psoriatic keratinocytes (for quantitations, see table 1). These proteins which correspond to IEF SPPs 4311 (40.3 kDa), 4003 (12.4 kDa), 5008 (11.9 kDa), 3012 (11.6 kDa), 6016 (11.6 kDa) and 1015 (10.1 kDa) in the normal human keratinocyte 2D gel protein database [16] (fig.3, lower panel) are synthesized, albeit at a reduced rate, by total keratinocytes from normal (fig.2) and normal appearing (uninvolved; fig.4A, table 1) skin from psoriatic patients. Cultured SV40 transformed human keratinocytes (K14) [11,12,18] and A431 cells synthesize only very low levels of these proteins (results not shown).

2D gel analysis of the [^{35}S]-methionine-labelled proteins released to the medium further indicated that these polypeptides are at least in part secreted. Fig.4C and D show IEF gels of [^{35}S]-methionine-secreted proteins from total epidermal keratinocytes from uninvolved (fig.4C; see also fig.4A) and psoriatic skin (fig.4D; see also fig.4B). Quantitative determination of the ratio of each individual protein to IEF 9105 in psoriatic vs normal (uninvolved) keratinocytes showed that this is similar to that observed for the cellular fraction (results not shown). The fraction of these proteins that is released to the medium is at present unknown.

There are a few lines of evidence suggesting that these highly up-regulated proteins are produced by keratinocytes. First, keratinocytes are by far the major cellular component of the psoriatic epidermis and the up-regulated proteins are relatively abundant as judged by Coomassie blue staining (results not shown). Second, 2D gels of proteins obtained from total epidermal keratinocytes of psoriasis skin do not show significant levels of plastin [19], a protein marker for lymphocytes and macrophages. The possibility that these proteins are produced by Langerhans cells cannot be excluded at the moment.

Small-molecular-weight proteins have previously been reported in psoriatic scales [20,21], but these are believed to correspond to degradation products of prekeratin polypeptides. Presently, all available evidence indicates that the proteins we have identified

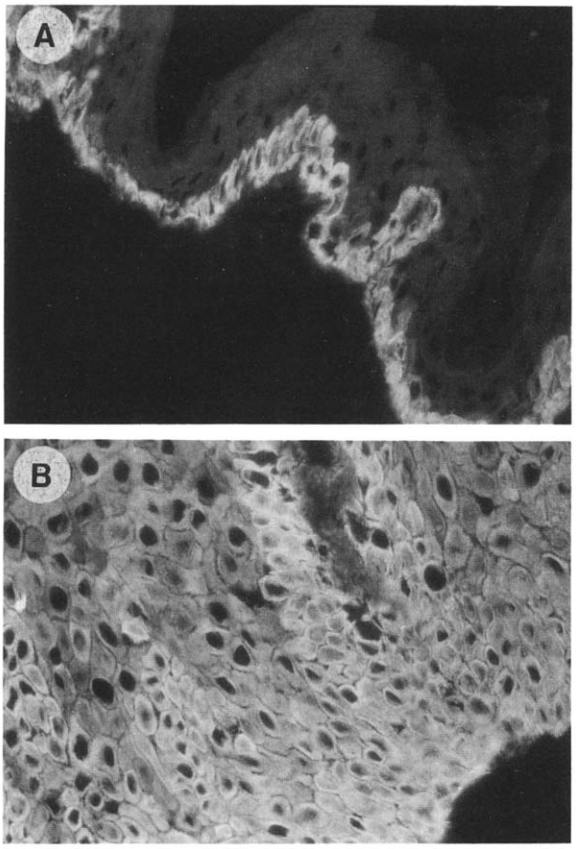

Fig.5. Immunofluorescence staining of methanol-fixed cryostat sections from (A) uninvolved and (B) psoriasis plaque reacted with mAB BG3C8.

are not derived from the proteolytic cleavage of abundant cellular proteins. They do not react with various keratin antibodies and their ratio is very similar from run to run (results not shown). Furthermore, these polypeptides seem to be different from IL-6 (21 kDa) and transforming growth factor α (5-8 kDa) [22 and references therein], cytokines which are known to stimulate the proliferation of human keratinocytes in culture [8,9,23]. The possibility that some of the low-molecular-weight up-regulated proteins may correspond to the psoriatic leukotactic factor [24] or to the anionic neutrophil-activating peptide [25] cannot be excluded at present.

At present, we do not know what the role of these proteins is in the pathophysiology of psoriasis. The fact, however, that they are at least in part secreted and are among the few polypeptides that are highly up-regulated in psoriatic keratinocytes warrant further biochemical studies. Furthermore, it will be interesting to determine whether these proteins are also up-regulated in other skin disorders.

Table 1

Proteins that are highly up-regulated in total human epidermal keratinocytes from psoriatic skin

Protein number in normal human keratinocyte 2D gel protein database[a]	Molecular mass (kDa)	Ratio[b] of psoriasis/ normal (uninvolved) keratinocytes
SSP 4311	40.3	14.67
SSP 4003	12.4	4.89
SSP 5008	11.9	9.93
SSP 3012	11.6	7.47
SSP 6016	11.5	5.12
SSP 1015	10.1	5.13

[a] See [16]. See also Figs.2 and 3 (lower panel)

[b] Spots cut from gels of psoriatic and uninvolved keratinocytes labelled for 14 h were counted in a liquid scintillation counter. Counts were normalized against IEF 9105 (see Figs.2 and 4)

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