Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1639 (2003) 80–86
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

Tryptophan hydroxylase expression in human skin cells

Andrzej Slominski[a,*], Alexander Pisarchik[a], Olle Johansson[b], Chen Jing[b], Igor Semak[c], George Slugocki[d], Jacobo Wortsman[e]

[a] *Department of Pathology, University of Tennessee Health Science Center, 930 Madison Ave., #519 Memphis, TN 38163, USA*
[b] *The Experimental Dermatology Unit, Department of Neuroscience, Karolinska Institute, 171 77 Stockholm, Sweden*
[c] *Department of Biochemistry, Belarus State University, Minsk, Belarus*
[d] *Department of Surgery, University of Tennessee Health Science Center, Memphis, TN 38163, USA*
[e] *Department of Internal Medicine, Southern Illinois University, Springfield, IL 62794, USA*

Received 25 March 2003; received in revised form 23 June 2003; accepted 21 July 2003

Abstract

We attempted to further characterize cutaneous serotoninergic and melatoninergic pathways evaluating the key biosynthetic enzyme tryptophan hydroxylase (TPH). There was wide expression of TPH mRNA in whole human skin, cultured melanocytes and melanoma cells, dermal fibroblasts, squamous cell carcinoma cells and keratinocytes. Gene expression was associated with detection of TPH immunoreactive species by Western blotting. Characterization of the TPH immunoreactive species performed with two different antibodies showed expression of the expected protein (55–60 kDa), and of forms with higher and lower molecular weights. This pattern of broad spectrum of TPH expression including presumed degradation products suggests rapid turnover of the enzyme, as previously reported in mastocytoma cells. RP-HPLC of skin extracts showed fluorescent species with the retention time of serotonin and *N*-acetylserotonin. Immunocytochemistry performed in skin biopsies localized TPH immunoreactivity to normal and malignant melanocytes. We conclude that while the TPH mRNA and protein are widely expressed in cultured normal and pathological epidermal and dermal skin cells, in vivo TPH expression is predominantly restricted to cells of melanocytic origin.

Keywords: Tryptophan hydroxylase; Serotonin; Skin; Melanocyte; Keratinocyte; Fibroblast

1. Introduction

Accumulated evidence indicates widespread detection of neuroendocrine elements in the skin [1]. Among these are the small molecules of the family of neurotransmitters, catecholamines and acetylcholine [2,3]. Most recently, we found other family components represented by the novel cutaneous expression of serotoninergic and melatoninergic elements [4,5]. The supporting evidence comprised expression of the genes coding for the corresponding biosynthetic enzymes, e.g., TPH, AANAT and HIOMT, together with the respective enzymatic activities. Moreover, intermediate products in the serotoninergic and melatoninergic pathways were also identified in melanoma cells and in immortalized keratinocytes [4,5]. Some of these findings were not unexpected since isolated detection of serotonin itself had been previously reported in normal human melanocytes and melanoma cells [6], and serotonin metabolism to *N*-acetylserotonin (NAS), 5-methoxytryptamine (5MTT) and possibly melatonin were demonstrated in hamster skin [7–9].

The rate-limiting step in the synthesis of serotonin is activity of tryptophan hydroxylase (TPH: EC1.14.16.4), the enzyme converting the aromatic amino acid L-tryptophan to hydroxytryptophan [10,11]. In humans, the TPH gene has been located at chromosome 11, spans 29 kb and 10 of the at least 11 exons code for the TPH protein [11–13]. The TPH nucleotide coding sequence has 1332 bp which encodes a protein of 444 amino acids (aa) with predicted molecular weight (mw) of 51 kDa. Prostranslational modifications generate species of 50–60 kDa mw with the most frequent form being 53 kDa [11–14]. Alternative splicing of the gene can yield isoforms with variable spliced 5′-untranslated regions, whereas alternative splicing of intron 11 produces a protein 22 aa longer (total of 466 aa) [12,13]. TPH monomers are organized into regulatory (N-terminal) and catalytic (C-terminal) domains; the latter includes a

* Corresponding author. Tel.: +1-901-448-3741; fax: +1-901-448-6979.
E-mail address: aslominski@utmem.edu (A. Slominski).

0925-4439/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0925-4439(03)00124-8

leucine zipper involved in the formation of the tetrameric holoenzyme of mw 220 kDa [11]. Fragment removal on deletional studies has shown that removing fragments from the regulatory domain of up to 106 aa will generate truncated proteins with higher TPH activity than the unmodified variant [11,14].

In the current study, we evaluated expression of TPH protein in the skin, as component of a serotoninergic/melatoninergic system. We tested whole human skin and a variety of skin cell lines. We also localized the TPH protein in situ in biopsies of human skin (normal and affected by invasive melanoma).

2. Materials and methods

2.1. Tissue

Human tissues consisted of non-lesional skin (23 biopsies from 14 patients and four large excisions from four patients) and pathologic skin from three patients with basal cell carcinoma and two patients involved by invasive malignant melanoma. The samples represented skin from face, corpus (back or chest) and upper and lower extremities from either male or female White patients with age ranging from 19 to 70 years old. The use of human tissues was approved by the Karolinska Institute, Stockholm, Sweden, and by the University of Tennessee Health Science Center (UTHSC) Committee on Research Involving Human Subjects, Memphis, TN.

2.2. Cell culture

Immortalized human epidermal melanocytes (PIG1) (gift of Dr. C. LePoole, Loyola Medical Center) were cultured in medium 154 (Cascade Biologicals Inc., Portland, OR) supplemented with 5% FBS, 13 μg/ml BPE, and 8 nM TPA, 1 μg/ml α-tocopherol, 0.6 ng/ml basic fibroblast growth factor, 1 μg/ml transferrin and 5 μg/ml insulin (all from Sigma) [4]. Normal human adult keratinocytes and dermal fibroblasts were obtained from Cascade Biologics. Keratinocytes were cultured in Cascade Epilife medium with Ca (0.06 mM) and Epilife Defined Growth Supplements (EDGS) (containing purified bovine serum albumin, purified bovine transferrin, hydrocortisone, recombinant human insulin-like growth factor type-1 (rhIGF-1), prostaglandin E2 (PGE2) and recombinant human epidermal growth factor (rhEGF). Dermal adult fibroblasts were cultured in Cascade 106 medium plus Cascade Low Serum Supplement (containing fetal bovine serum, 2% v/v; hydrocortisone, 1 μg/ml; human epidermal growth factor, 10 ng/ml; basic fibroblast growth factor, 3 ng/ml; and heparin, 10 μg/ml and penicillin/streptomycin/amphotericin B solution). Immortalized HaCaT keratinocytes and human C4-1 squamous cell carcinoma cells were cultured in DMEM media, supplemented with 10% fetal calf serum (FCS) (Gibco) and 1 × antibiotic/antimicotic mixture containing 1000 U/ml of penicillin, 0.1 mg/ml of streptomycin and 0.25 μg/ml of amphotencin B (Sigma). The SKMEL188 melanoma cell line was cultured in Ham's F10 medium plus 10% FBS. Other melanoma lines established from radial growth phase (WM 35 and SBCE2), vertical growth phase (WM 98 and WM 1341D) and metastasis (WM 164) (gift of Dr. M. Herlyn, Wistar Institute, Philadelphia, PA) were maintained in DMEM plus insulin (1 μg/ml) in the presence of 5% $C0_2$ as described previously. Culture media were routinely supplemented with 10% fetal bovine serum (Gibco) and antibiotics (Sigma). Media were changed every second day.

2.3. Reverse transcription polymerase chain reaction assays (RT-PCR)

Total RNA was extracted using the Trizol isolation kit (Gibco-BRL, Gaithersburg, MD) and the synthesis of first-strand cDNA was performed using the Superscript preamplification system (Gibco-BRL) with oligo (dT) as the primer [4].

C-terminus of human TPH was amplified by nested PCR using following primers for the first round of amplification: forward-AGCCAGATACCTGCCATGAAC (P100), reverse-GCTGCAGCTCATTCATGGCAC (P101). An aliquot of PCR mixture from the first round of amplification was transferred to a new tube and a second round of PCR was conducted. Primer sequences for the second round of PCR were: forward-CCAAGAAATTGGCTTGGCTTCTC (P106); reverse-TGCTCTTGGTGTCTTTCAGGATC (P107). The reaction mixture (25 μl) contained 2.5 mM $MgCl_2$, 2.5 of each dNTP, 0.4 μM of each primer, 75 mM Tris–HCl (pH 8.8), 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20 and 1.25 U of Taq polymerase (Promega). The mixture was heated to 94 °C for 2.5 min and then amplified for 30 cycles as specified: 94 °C for 30 s (denaturation), 65 °C for 45 s (annealing) and 72 °C for 1 min (extension).

N-terminal region of TPH was amplified in a single round of PCR by primers TTGAAGACAATAAGGAGAACAAAG (P323) and TCTAGTTCAGATCCATACATCAG (P324). Amplification conditions for all skin samples were stringent and identical to those described above except that annealing temperature was set at 56 °C.

The fragment spanning protein coding exons 1 through 10 was amplified by nested PCR. The first round of PCR was performed with primers P323 and P101. Primers P323 and P107 were used for the second round. The mixture was heated to 94 °C for 3 min and then amplified for 30 cycles as specified: 94 °C for 30 s (denaturation), 55 °C for 40 s (annealing) and 72 °C for 2 min (extension).

Amplification products were separated by agarose electrophoresis and visualized by ethidium bromide staining according to the standard protocol used in our laboratory [4,5]. PCR products were identified and excised from the agarose gel and purified by GFX PCR DNA and gel band purification kit (Amersham Pharmacia Biotech). PCR prod-

ucts were cloned in pGEM-T easy vector system (Promega) and purified by plasmid purification kit (QIAGEN). Sequencing was performed in the Molecular Resource Center at the University of Tennessee HSC (Memphis) using Applied Biosystems 3100 Genetic Analyzer and BigDye™ Terminator Kit.

2.4. TPH detection by Western blot analysis

Briefly, melanoma cells were detached in Tyrode's solution (138 mM NaCl, 2.7 mM KCl, 3.14 mM $Na_2PO_4H_2O$, 2.31 mM Na_3 C_6 H_5O_7 $2H_2O$, 0.1% D-glucose) plus 1 mM EDTA, whereas melanocytes, keratinocytes, squamous cell carcinoma and fibroblasts were trypsinized. Cell suspensions were centrifuged at 200 × *g* for 10 min at 4 °C. The resulting cell pellets were washed with PBS and frozen in − 70 °C. For protein isolation, frozen cell pellets were solubilized by pipetting into an iced buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl, 15% glycerol, 1% Triton X-100, 120 μg/ml leupeptin, 3 μM pepstatin and 3 mM amino-ethyl benzene sulfonyl fluoride (AEBSF). Cellular homogenates were centrifuged at 16,000 × *g* for 10 min at 4 °C, and the supernatants were removed and stored at − 80 °C for further analysis. Separate aliquots of 5 μl were used for protein determination by Micro protein Kit (Sigma). Fifty micrograms of protein were loaded on 12% SDS polyacrylamide gel, transferred to immobilion-p (polivinylidene difluoride) membrane (Millipore Corp., Bedford, MA) for 3 h at 4 °C and blocked for 4 h at room temperature in 5% nonfat powdered milk in TBST (50 mM Tris, pH 7.5, 150 mM NaCl, 0.01% Tween-20). Immunodetections of the TPH protein were performed after independent overnight incubations at 4 °C (dilution 1:1000) with either affinity-purified polyclonal rabbit anti-TPH antibody (dilution 1:1000; lot #19030270) or sheep anti-TPH primary antibody (dilution 1:500; lot #22010680) as the primary antibody (Chemicon, Temecula CA). The next day, membranes were washed twice in TBST for 10 min. Goat anti-rabbit IgG or bovine anti-sheep IgG coupled to horseradish peroxidase were respectively used as a secondary antibody (dilution 1:4000, 1 h) (Santa Cruz Biotechnology). Membranes were washed twice in TBST and once in TBS. Bands were visualized by ECL reagent according to the manufacturer's instructions (Amersham Pharmacia Biotech).

2.5. Immunohistochemistry

Paraffin sections were deparaffinized, rehydrated and processed in citrate buffer for antigen retrieval by microwave treatment. After hydrogen peroxide quenching for endogenous peroxidase activity, and blocking of nonspecific binding with normal swine serum (Dako A/S, Glosterup, Denmark), the sections were incubated overnight at 4 °C with primary polyclonal antibody (1:200; rabbit anti-TPH; Chemicon International Inc.). The tissue sections were then washed and treated with biotinylated swine anti-rabbit serum (1:200; Dako). After washing, the sections were incubated with avidin biotin–peroxidase complex (Vector Laboratories), washed and visualized with 3-amino-9-ethyl-carbazole as enzyme substrate, and hematoxylin as counterstain.

Indirect immunofluorescence stains were performed according to standard protocols [6]. Briefly, serial cryostat sections (14 um) were obtained and processed as described below. Rabbit polyclonal antibodies to TPH (1:200; Chemicon), mouse monoclonal antibodies to HLA-DR (to stain dendritic cells of immune origin) (1:200; Dako) mouse monoclonal antibodies to melanocyte-associated antigen NKI-beteb (1:80; Sanbio BV, Am Uden, the Netherlands) were used for the indirect immunofluorescence technique, in either single- or double-labeling experiments. Tissue sections, kept in a humid atmosphere, were incubated overnight at 4 °C, with the abovementioned antisera; after rinsing in phosphate-buffered saline, sections were incubated for an additional 30 min at 37 °C in tetramethylrhodamine-isothiocyanate (TRITC)-conjugated donkey anti-rabbit IgG (1:160; Jackson, West Grove, PA), or fluorescein-isothiocyanate (FITC)-conjugated donkey anti-mouse IgG (1:160, Jackson), rinsed and mounted. All antisera were diluted in 0.3% Triton X-100. A control test for nonspecific binding of the conjugated antibodies was represented by omission of the primary antibody. To test for secondary antiserum cross-reaction in the double-staining experiments, sections were incubated with the rabbit antibodies, and by FITC-conjugated donkey anti-mouse IgG and mouse antibodies followed by TRITC-conjugated donkey anti-rabbit IgG. Visual inspection and photography were performed with a Nikon Microphot-FXA equipped with dark-field optics.

2.6. Reverse phase high performance liquid chromatography (RP-HPLC)

A fluorimetric detection system for serotonin was used as described previously [4]. Briefly, the skin was processed by homogenization and the centrifuged supernatants subjected to HPLC in a system equipped with a Novapak C_{18} reverse-phase column (100 × 5 mm, i.d.) and fluorometric detector (Waters). Detector calibration was set at excitation and emission wavelengths of 285 and 360 nm, respectively. Elution was carried out isocratically at ambient temperature with a flow rate of 1.5 ml/min for the different mobile phases according to the amine substrate being evaluated. The mobile phase contained 4 mM sodium 1-octanesulfonate as ion-pairing agent, 50 mM ammonium formate (pH 4.0) versus methanol (80:20) for detection of serotonin. The elution peak of serotonin was identified by retention time, and the identity verified by co-elution with the authentic standard.

3. Results and discussion

When using primers located at exons coding for the C-terminal (catalytic domain) of TPH (Fig. 1A) we detected

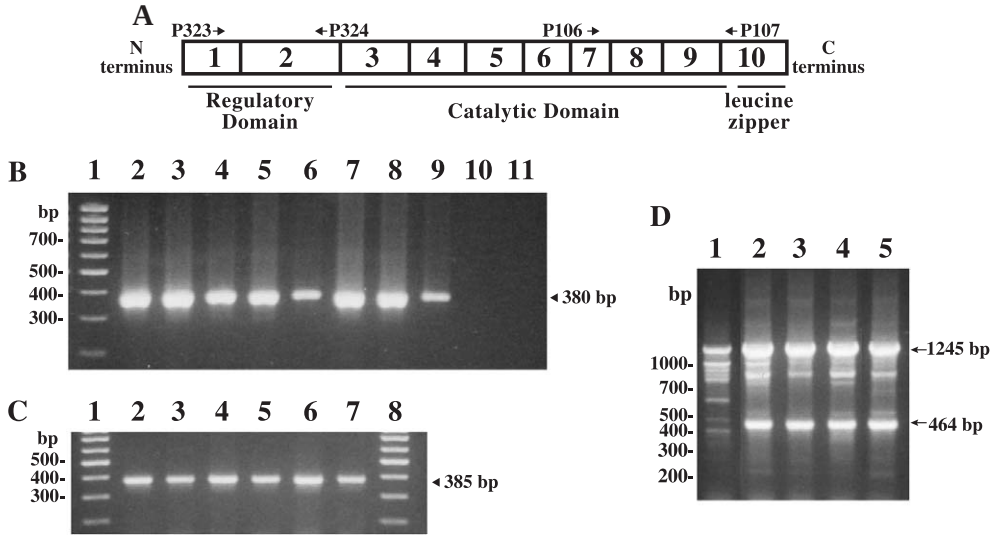

Fig. 1. RT-PCR of human TPH. (A) Structure of the coding region of the human TPH gene. Open boxes and corresponding arbitrary numbering of the exons (1 to 10) relate only to the coding region. Note that the TPH gene is known to contain at least one more exon in the nontranslated region [12]. Arrows indicate primer positions. (B) RT-PCR of the catalytic domain. DNA ladder, 1; human pituitary, 2; adrenal gland, 3; myometrium, 4; human skin, 5; melanoma SKMEL-88, 6; basal cell skin carcinomas, 7–9; HaCaT, 10; RT-control, 11. (C) RT-PCR of the regulatory domain: DNA ladder, 1,8; immortalized melanocytes, 2; melanomas SBCE2, 3; WM35, 4; WM164, 5; WM1341D, 6; WM98, 7. (D) Long-range PCR of human TPH: HaCaT keratinocytes, 1; melanomas SBCE2, 2; WM35, 3; WM98, 4; WM1341, 5. On the left, position and size (bp) of the DNA ladder are listed.

previously expression of TPH mRNA by RT-PCR in cultured epidermal and follicular keratinocytes and melanocytes, dermal and hair papilla fibroblasts with exception of immortalized HaCaT cells [4]. We repeated these experiments and found similar expression in whole normal and pathologic skin, cultured squamous cell carcinoma C4-1 and melanoma lines but not in HaCaT keratinocytes (Fig. 1B). TPH RNA was also detected in non-skin tissues represented by pituitary, adrenal gland and myometrium. Re-testing of the original samples with primers located instead at exons coding for the N-terminus (regulatory domain) of the enzyme showed almost uniform expression of TPH mRNA (Table 1; representative panel of several experiments is shown in Fig. 1C), which this time included immortalized HaCaT keratinocytes (not shown); the sole exception was represented by the single melanoma cell line (SKMEL-188) (Table 1). In addition, in four melanoma specimens, we also amplified the 1245-bp-long fragment of TPH mRNA spanning the coding exons 1 through 10 that corresponded to the published sequence of human TPH (Fig. 1D; GenBank accession #X52836). This full-length transcript was also detected in the HaCaT cells (Fig. 1D, lane 1). However, the observed sequence in HaCaT keratinocytes contained several mutations (GenBank accession #AY196344 and AY196345) leading to amino acid substitutions: one in the regulatory domain (F77L) and one in the catalytic domain (F377P). The presence of the above mutations in the HaCaT TPH sequence together with technical problems with the detection of C-terminal TPH mRNA in the same cell line suggest an additional mutation in the binding site of primer P101, thus providing an explanation for our failure to amplify the C-terminus in these cells. The additional 464-bp-long band shown in Fig. 1D represents an aberrantly spliced form of TPH (GenBank accession #AY196346). Since this form contains a stop codon, it is unlikely to have significant enzymatic activity. Thus, skin cells express both

Table 1
Expressions of TPH gene and TPH immunoreactive proteins in skin cells

Specimen	TPH antigen	TPH mRNA (C-terminus)	TPH mRNA (N-terminus)	TPH mRNA (full-length)
Whole skin	+	+	+	ND
Cultured cells				
SBCE2 melanoma cells	+	+	+	+
WM35 melanoma cells	+	+	+	+
WM164 melanoma cells	+	+	+	ND
SKMEL-188 melanoma cells	+	+	–	–
WM1341 melanoma cells	+	+	+	+
WM98	+	+	+	+
PIG-1 melanocytes	+	+	+	ND
Dermal fibroblasts	+	+	+	ND
C4-1 squamous cell carcinoma	+	+	+	ND
HaCaT keratinocytes	+	–	+	+
Adult epidermal keratinocytes	+	+	+	ND

The results represent a summary of several independent experiments. Present (+); absent (–); not done (ND).

the correct TPH transcript and aberrant TPH isoforms. At least in HaCaT keratinocytes, mutations in the regulatory and catalytic domains are the most likely cause of undetectable enzymatic activity in this cell line.

Several independent Western blotting experiments of cytoplasmic extracts from skin homogenates and cultured skin cells identified proteins specifically detected with two different anti-TPH antibodies (rabbit and sheep) (Fig. 2) (Table 1). Thus, the specific 55–60 kDa protein was visualized after treatment with anti-TPH sheep antibodies (arrow) in normal epidermal keratinocytes, immortalized HaCaT keratinocytes, squamous cell carcinoma, immortalized melanocytes, dermal fibroblasts and melanoma cells (Fig. 2A, upper panel); corresponding bands were absent in control samples incubated only with secondary antibodies (Fig. 2A, lower panel). Sheep anti-TPH antibodies also brought out additional proteins of higher (85–90 kDa) and lower (33–35, 27 and 20 kDa) mw (Fig. 2A); whereas rabbit anti-TPH antibodies emphasized TPH-like antigens of lower mw (33–35 and 20 kDa) as prominent species, as well as low lower levels of 55–60 kDa and 80–90 kDa mw species (Fig. 2B). Thus, the protein of expected size, 55–60 kDa, was detected by this antibody in normal epidermal keratinocytes, immortalized HaCaT keratinocytes, squamous cell carcinoma, immortalized melanocytes and melanoma cells. In the case of dermal fibroblasts, the discrepancy between results with sheep and rabbit antibodies is probably explained by differences in the epitope(s) recognized by respective anti-TPH antibodies or by rapid degradation of the enzyme. Prominent expression of the 48 kDa protein was found in whole human skin after treatment with the rabbit antibody (Fig. 2B), and that also showed an 80 kDa protein in SKMEL188 melanoma (not shown). Thus, using two different antibodies, we detected by Western blots THP immunoreactivities of expected size together with higher or lower mw forms in a variety of skin samples.

The mw of newly translated TPH changes after post-translational modification [11]. This is consistent with our detection of the 48 kDa form as the prominent species in skin extracts, and of a protein of 55–60 kDa in normal and malignant skin cells (Fig. 2). Variants with higher and lower mw (TPH degradation products) have also been described [16]. For example, in mastocytoma cells ubiquitination of TPH can generate higher mw species of 80–93 kDa [16]. Further, studies in mastocytoma cells have shown that TPH undergoes very fast turnover driven by proteasomes leading to the degradation of native TPH into species of lower mw [17]. Similar mechanisms may operate in skin cells, where the appearance of high mw TPH-like species could represent ubiquitinated TPH, whereas low mw TPH-like species could represent rapid degradation (Fig. 2). In vitro experiments, of deletion of different portions of the human TPH gene have shown more enzymatic activity in a protein lacking the N-terminus regulatory domain with mw of approximately 35 kDa, than in the full-length form [11,15]. Thus, the 35 kDa species we detected in many skin

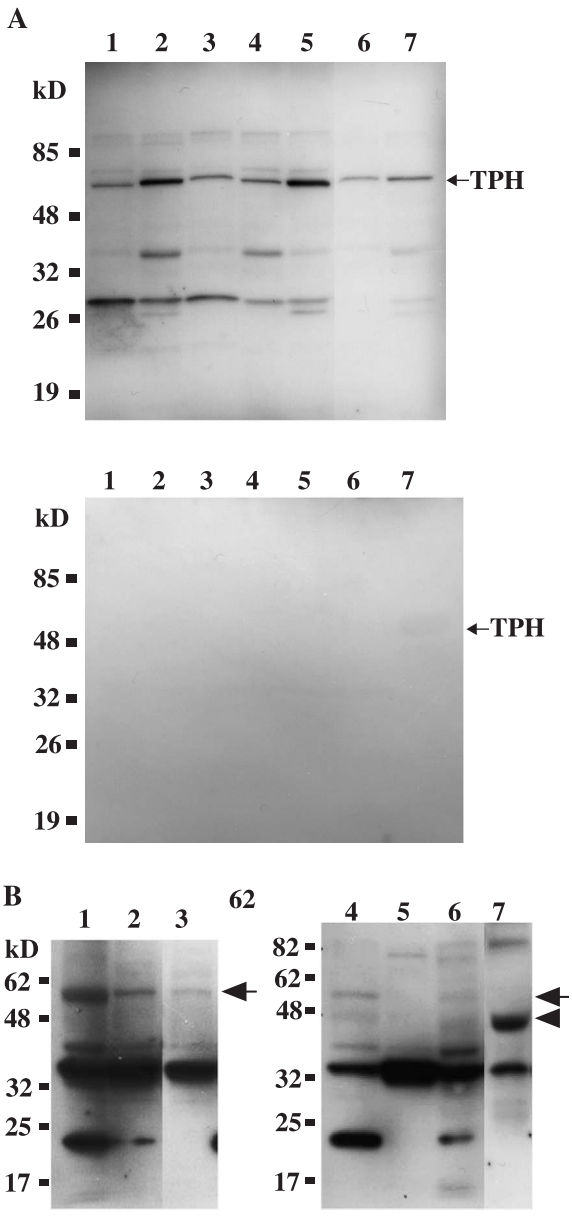

Fig. 2. Detection of TPH immunoreactive proteins by Western blot analysis. (A) Immunodetection using sheep anti-TPH antibodies (upper panel). Lower panel presents blots incubated with secondary antibody only. Mw marker (kDa), on the right; HaCaT immortalized keratinocytes, 1; normal epidermal keratinocytes, 2; C4-1 squamous cell carcinoma cells, 3; dermal fibroblasts, 4; immortalized melanocytes, 5; melanoma SKMEL188, 6; and WM164, 7. Arrow indicates TPH-like immunoreactivity of 55–60 kDa. (B) Immunodetection using rabbit anti-TPH antibodies: HaCaT immortalized keratinocytes, 1; normal epidermal keratinocytes, 2; immortalized melanocytes, 3; C4-1 squamous cell carcinoma cells, 4; dermal fibroblasts, 5; melanoma WM35, 6; human skin, 7. Arrow indicates TPH-like immunoreactivity of 55–60 kDa; while arrowhead indicates TPH-like immunoreactivity of approximately 48 kDa.

samples could represent C-terminus products of TPH catabolism still having the catalytic domain; and the 27 and 20 kDa species could represent either proteolytically cleaved N-terminus TPH regulatory domain or a product of further degradation of 35 kDa protein(s). Since alternative splicing

has also been reported for the TPH gene [12,13], part of the diversity in TPH-like immunoreactivity mw could additionally be due to translation of alternatively spliced TPH mRNA.

Immunohistochemical analysis of paraffin-embedded pathologic skin showed intense TPH labeling of normal melanocytes in the basal layer as well as in the spinous and granular layers. Strong stain for TPH was also seen in melanoma cells, whether intraepidermally or in the dermal (invasive) compartment (Fig. 3A). TPH immunoreactive cells of normal skin were distributed sparsely throughout the stratum basale separated by three to five epidermal basal cells in between (not shown). The TPH signal was further localized to the cellular cytoplasm compartment. Interestingly, the cells staining positive appeared dendritic in nature, with rich cytoplasm and short, thin processes that also stained with NKI-beteb antibodies. We identified those cells as melanocytes, which was confirmed by their double labeling with antibodies against TPH and NKI-beteb (Fig. 3B). Potentially all skin cells can express TPH (cf. cell culture experiments), nevertheless, in human skin, in vivo TPH is expressed predominantly in normal and malignant melanocytes. Whether TPH could represent a potential therapeutic target in melanoma and/or pigmented disorders is certainly an interesting possibility, especially when considering that it represents a first regulatory point of serotoninergic and melatoninergic systems.

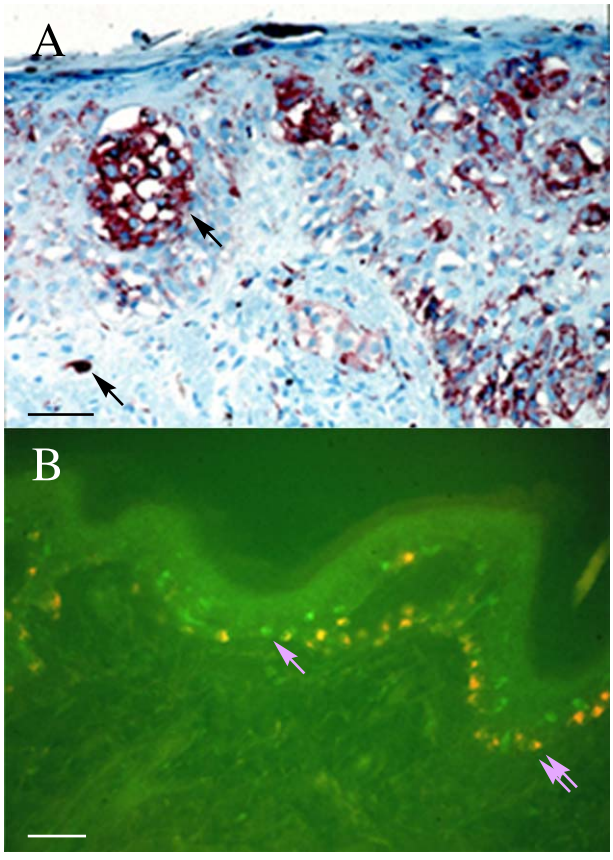

Fig. 3. Detection in situ of TPH-positive cells in the human skin. (A) Immunohistochemistry of biopsy from skin involved with melanoma. Arrows indicate TPH-immunoreactive cells in both the epidermis and dermis. Bar indicates 50 μm. (B) Immunofluorescence double-staining in normal human skin. Red: cells positive for NKI-beteb antigen; green: cells positive for TPH; orange: double-labeled cells. Singe arrow indicates TPH-immunoreactive cells. Double arrows indicate double-labeled NKI-beteb and TPH-positive cells in the stratum basale of the epidermis. Bar indicates 50 μm.

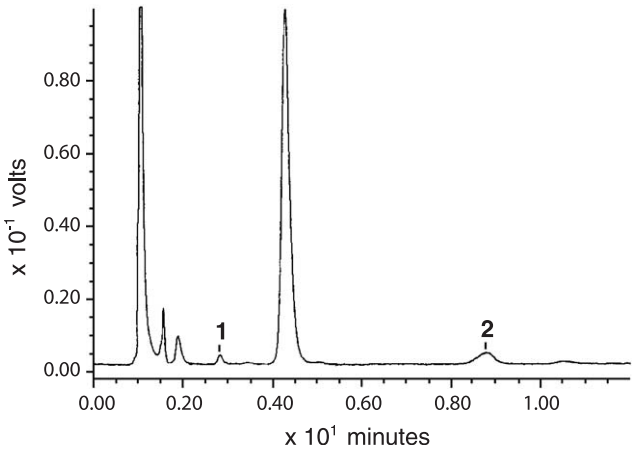

Fig. 4. Representative HPLC chromatograms of the skin. Endogenous fluorescence is present at the elution position of standards corresponding to NAS (1) and serotonin (2).

The co-localization of the enzyme (present study) with the serotonin antigen [6] implies active metabolism in situ, from tryptophan to serotonin. Indeed, RP-HPLC analysis of human skin extracts showed clear fluorescent peaks that eluted at retention times corresponding to the serotonin and NAS standards, suggesting cutaneous metabolism of tryptophan to serotonin and NAS, with storage of both amines (Fig. 4). These results are also consistent with our previous studies in cultured melanoma cells, where we not only detected TPH activity but also identified OH-Trp, serotonin and NAS by LC/MS [5].

Serotonin has a number of effects in the skin that include pro-edema, vasodilatory, pruritogenic and proinflammatory activities, as well as growth factor actions. Since these are common components of a number of skin disorders, it is possible that locally produced serotonin could participate in their pathogenesis. Serotonin local synthesis and cellular localization could thus become of great importance in the diagnosis and management of cutaneous pathology.

In the current study, we show TPH gene and protein expression in the human skin and its cellular localization with predominance to normal and malignant melanocytes. Western blot analysis and RT-PCR tests suggest alternative splicing of the gene and potentially rapid turnover of the enzyme in skin cells cultured in vitro. Thus, cultured skin cells represent excellent models for the study of differential expression of TPH isoforms, as well as for the cellular processing of the protein product. These findings strongly support the full expression of a novel serotoninergic system in the skin. This system appears to be secluded in vivo to a

tightly regulated cutaneous environment at a highly compartmentalized level.

In conclusion, the enzyme TPH is widely expressed in cultured skin cells; in vivo, it is instead localized, predominantly to normal and malignant melanocytes.

Acknowledgements

The work was supported in part by grants from Vitiligo Foundation, Center of Excellence in Genomics and Bioinformatics and Center of Excellence in Connective Tissue Diseases, UTHSC to A.S. and the Cancer and Allergy Foundation (Cancer-och Allergifonden) and funds from the Karolinska Institute to O.J. We also thank Marianne Ekman for skilful technical assistance.

References

[1] A. Slominski, J. Wortsman, Neuroendocrinology of the skin, Endocr. Rev. 21 (2000) 457–487.
[2] K.U. Schallreuter, K.R. Lemke, M.R. Pittelkow, J.M. Wood, C. Korner, R. Malik, Catecholamines in human keratinocyte differentiation, J. Invest. Dermatol. 104 (1995) 953–957.
[3] S.A. Grando, R.M. Horton, The keratinocyte cholinergic system with acetylcholine as an epidermal cytotransmitter, Curr. Opin. Dermatol. 4 (1997) 268–462.
[4] A. Slominski, A. Pisarchik, I. Semak, T. Sweatman, J. Wortsman, A. Szczesniewski, G. Slugocki, J. McNulty, S. Kauser, D.J. Tobin, C. Jing, O. Johansson, Serotoninergic and melatoninergic systems are fully expressed in human skin, FASEB J. 16 (2002) 896–898 (full text FASEB J. (April 23, 2002) 10.1096/fj.0.1-0952fje).
[5] A. Slominski, I. Semak, A. Pisarchik, T. Sweatman, A. Szczesniewski, J. Wortsman, Conversion of L-tryptophan to serotonin and melatonin in melanoma cells, FEBS Lett. 511 (2002) 102–106.
[6] O. Johansson, P.-Y. Liu, L. Bondesson, K. Nordlind, M.J. Olsson, W. Lontz, A. Verhofstad, Y. Liang, S. Gangi, A serotonin-like immunoreactivity is present in human cutaneous melanocytes, J. Invest. Dermatol. 111 (1998) 1010–1014.
[7] S. Gaudet, A. Slominski, M. Etminan, D. Pruski, R. Paus, M.A.A. Namboodiri, Identification and characterization of two isozymic forms of arylamine *N*-acetyltransferase in Syrian hamster skin, J. Invest. Dermatol. 101 (1993) 660–665.
[8] A. Slominski, J. Baker, T. Rosano, L.W. Guisti, G. Ermak, M. Grande, S.J. Gaudet, Metabolism of serotonin to *N*-acetylserotonin, melatonin, and 5-methoxytryptamine in hamster skin culture, J. Biol. Chem. 271 (1996) 12281–12286.
[9] A. Slominski, A. Pisarchik, I. Semak, T. Sweatman, A. Szczesniewski, J. Wortsman, Serotoninergic system in hamster skin, J. Invest. Dermatol. 119 (2002) 934–942.
[10] I.P. Kema, E.G.E. de Vries, F.A.J. Muskiet, Clinical chemistry of serotonin and metabolites, J. Chromatogr., B. 747 (2000) 33–48.
[11] S.M. Mockus, K.E. Vrana, Advances in the molecular characterization of tryptophan hydroxylase, J. Mol. Neurosci. 10 (1998) 163–179.
[12] G.-A. Wang, S.L. Coon, S. Kaufman, Alternative splicing at the 3′-cDNA of human tryptophan hydroxylase, J. Neurochem. 71 (1998) 1769–1772.
[13] S. Boularand, M.C. Darmon, J. Mallet, The human tryptophan hydroxylase gene. An unusual splicing complexity in the 5′-untranslated region, J. Biol. Chem. 270 (1995) 3748–3756.
[14] H. Nakata, F. Hitoshi, Tryptophan 5-monooxygenase from mouse mastocytoma P815. A simple purification and general properties, J. Biochem. 124 (1982) 595–601.
[15] X.-J. Yang, S. Kaufman, High-level expression and deletion mutagenesis of human tryptophan hydroxylase, Biochemistry 91 (1994) 6659–6663.
[16] M. Kojima, K. Oguro, K. Sawabe, Y. Iida, R. Ikeda, A. Yamashita, N. Nakanishi, H. Hasegawa, Rapid turnover of tryptophan hydroxylase is driven by proteasomes in RBL2H3 cells, a serotonin producing mast cell line, J. Biochem. 127 (2000) 121–127.
[17] H. Hasegawa, M. Kojima, K. Oguro, N. Nakanishi, Rapid turnover of tryptophan hydroxylase in serotonin producing cells: demonstration of ATP-dependent proteolytic degradation, FEBS Lett. 368 (1995) 151–154.