Short Communication

Suprabasal Overexpression of the hsRPB7 Gene in Psoriatic Epidermis as Identified by a Reverse Transcriptase-Polymerase Chain Reaction Differential Display Model Comparing Psoriasis Plaque Tissue with Peritonsillar Mucosa

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In psoriasis an etiopathogenetic vicious circle is nowadays hypothesized that the disease is triggered by skin-specific autoantigen structures, the expression and accessibility of which are positively correlated with the intensity of the hyperproliferation and inflammation in the epidermopapillary compartment driven by autoreactive T cells. Despite the close microanatomical relation between skin and mucosa, clinicians have always been intrigued by the observation that psoriatic affection of the mucosa, if at all existing, is only seen as very rare events in the lips and tongue sparing buccopharyngeal sites. This prompted us to establish an experimental model system comparing psoriatic-involved skin and peritonsillar mucosa from tonsillectomies by a reverse transcriptase-polymerase chain reaction/differential display strategy. Among more than 60 cDNA species to be displayed in psoriasis, but missing in peritonsillar mucosa, one species was identified as coding for the RNA polymerase II A seventh subunit (hsRPB7 gene) as a most critical factor for DNA to RNA transcription. Immunohistochemistry showed a hitherto unknown, distinctive pattern of hsRPB7 expression that was 1) tissue type-dependent with a surplus in skin keratinocytes and a near absence in peritonsillar mucosa, 2) tightly regulated by the keratinocyte differentiation process with a sharp suprabasal up-regulation in contrast to a basal down-regulation, and 3)

substantially augmented in psoriatic-involved skin as compared to normal and psoriatic uninvolved skin. Keratinocytes of actinic keratoses also showed a strong hsRPB7 expression that however did not strictly spare the basal cell layer presumably reflecting the disturbed intraepidermal stratification because of the premalignant status of these precancerous lesions. (Am J Pathol 2001, 158:367–372)

The etiology of psoriasis is still unknown.^{1–7} But there is a lot of at least indirect clinical and experimental evidence that speaks in favor of a predominantly immunological quality of its pathogenesis. Nowadays, autoantigen-directed mechanisms intermingled with microbial (super)-antigen-driven immune-activations are hypothesized to play major roles in psoriasis, with the primary relevance of T-cell actions prevailing over antibody-mediated processes.^{4–11} In this pathogenetic concept it is a matter of current controversial debates if such putatively expressed HLA-restricted autoantigens are recognized by CD4⁺ or CD8⁺ lymphocytes.^{2,3,12,13} Nevertheless, an alternate etiological concept of psoriasis as a disease with an antigen-independent pathogenesis has still to be taken into careful consideration. Only very recently, the possible crucial involvement of components of the innate immune systems including natural killer characteristics of T cells has been brought to the awareness of the scientific community.^{4,5,14} Moreover, the obvious clinical diver-

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sity of psoriasis and its variants lends support to the notion that heterogeneous pathomechanism may co-exist, as well as combinations thereof.

Whether the oral mucosa can be specifically affected by psoriasis is an open question.¹⁵⁻¹⁸ This is partly because of the general observation, that signs of a possible psoriatic involvement of the oral mucosa are only seen in rare cases, mostly in conjunction with pustular skin manifestations of psoriasis.¹⁹⁻²² In such cases, the lips may show an exfoliative psoriatic cheilitis, and usually the tongue presents with an exfoliative areata linguae (ie, a so-called geographical tongue or benign migratory glossitis). The latter manifestation is histologically characterized by intraepithelial microabscesses of neutrophilic leukocytes also known as a quite pathognomonic feature of psoriatic skin affection.^{19,20,23} Attempts have been made to explain the rarity of this still questionable psoriatic involvement of oral mucosa by pointing to the fact that in this tissue compartment the epithelial proliferation rate reaches under physiological conditions already such a high level that hypothetically may not be further increased in a psoriasis-typical manner.²¹ But this explanation seems to be insufficient to a certain extent, as it relates only to epithelial hyperproliferation without addressing the phenomenon that an inflammatory infiltrate as another histological hallmark of psoriatic skin manifestation is usually missing in the oral mucosa of psoriasis patients. Therefore an alternate hypothesis might be raised postulating: 1) the missing expression or accessibility of putative psoriasis-relevant autoantigens or 2) the lack of psoriasis-determining antigen-independent alterations of gene expression, respectively, in oral mucosa as possible decisive reasons for its common noninvolvement in the psoriatic disease process. Most interestingly, the manifestation of psoriasis in a split-skin graft transplanted into the oral cavity has recently been reported emphasizing the crucial pathogenetic role of the epidermo-dermal compartment in psoriasis.²⁴

Given these considerations, we have established an experimental model comparing directly the gene expression between psoriatic plaque tissue and oral peritonsillar mucosa by a differential display/reverse transcriptase polymerase chain reaction (DD/RT-PCR) approach. As reported herein, this strategy led to the identification of more than 60, until now unknown, cDNA species up-regulated in the psoriatic plaque as compared to the mucosa background. Additionally, this comparison showed an overexpression of the transcription-related *hsRBP7* gene in psoriasis, which was analyzed by immunohistochemistry in detail.

Materials and Methods

Nonradioactive Differential Display RT/PCR

Our recent nonradioactive modification²⁵ of the original DD/RT-PCR protocol²⁶ was used as a technique for an optimized visualization and PCR reamplification of differentially displayed cDNA bands detected by silver staining. In brief, tissue specimens from skin lesions of plaque

psoriasis, normal skin, and tonsillectomy-derived peritonsillar mucosa²⁷ were frozen in liquid nitrogen and then homogenized on ice (Polytron PT3000, Kinematica AG). Total RNA was isolated by the standard guanidinium isothiocyanate method (RNAzol B), and mRNA was purified by a single run through an oligo(dT)-cellulose spun column (Pharmacia). The cDNA resulting from reverse transcription with anchored primers T₁₂AN was used as a template in a 20- μ l PCR reaction containing 1 μ mol/L of the T₁₂AN primer and an arbitrary 10-mer primer²⁸ each, 2.5 U AmpliTaq DNA polymerase (Perkin Elmer), 200 μ mol/L dNTP, and 2 mmol/L MgCl₂. Kinetics of the PCR reactions were set to 10 minutes at 94°C, 41 cycles of 30 seconds at 94°C, 30 seconds at 42°C, and 60 seconds at 72°C with a 10 minutes termination step at 72°C. The PCR-amplified cDNA species were separated electrophoretically on polyester film-backed 10% polyacrylamide gels (CleanGel, ETC) under nondenaturing flatbed conditions at 15°C. The cDNA bands were detected by an optimized silver staining as described in detail earlier.²⁵

From the differential display gels those cDNA bands were cut out that were identified for psoriatic tissue but found to be missing for normal oral mucosa when comparing corresponding lanes running side-by-side. The cDNA polyacrylamide material was intensely chopped by scalpel cuttings and squashed by a micropestle. The extraction was performed in a 0.5 mol/L ammonium acetate/1 mmol/L ethylenediaminetetraacetic acid solution (pH 8.0) at 37°C overnight under vigorous shaking. After centrifugation, the supernatant was glass microfiber-filtered (2- μ m pore size, Whatman) to remove polyacrylamide remnants. The cDNA was ethanol-precipitated, redissolved, ethanol-reprecipitated, and washed, as well as vacuum-dried for the subsequent PCR reamplification under the conditions given above.²⁵ The PCR reamplification product was run in a 0.8% low-melting agarose gel, subcloned (pCR2.1-TOPO; Invitrogen) and submitted to a nonradioactive cycle-sequencing reaction (Thermo Sequenase, Amersham). Sequencing was performed by using UV-polymerized gels (Repro Gel Long Read) in conjunction with an automated sequencer (AL-Fexpress, Pharmacia).

Immunohistology

For this purpose, biopsies of psoriatic plaques, peritonsillar mucosa, uninvolved psoriatic, and normal skin as well as seborrheic and actinic keratoses ($n = 3$ to 6 donors each) were placed in frozen specimen-embedding medium (catalog no. 6769006; Shandon, Pittsburgh, PA), quickly frozen on a freezing block at -55 to -60°C, cut into 5- μ m slices using a microtome, and fixed with acetone (4°C, 10 minutes). The slides were incubated with a murine monoclonal anti-RBP7-antibody (dilution 1:500; BAb Co., Richmond, CA)^{29,30} with a subsequent detection by an alkaline phosphatase Vectastain ABC kit (catalog no. AK-5002; Vector Laboratories Inc., Burlingame, CA) in conjunction with a Vector red substrate kit (catalog no. SK-5100, Vector Laboratories, Inc.). Endogenous alkaline phosphatase activity was blocked by add-

Table 1. Panel of 65 Novel cDNA Species Found to Be Up-Regulated in Psoriatic-Involved Skin as Compared to Normal Peritonsillar Mucosa when Performing a Differential Display/RT-PCR Analysis

AF126046: 151 bp	AF136806: 235 bp	AF136805: 267 bp	AF143341: 328 bp
AF126047: 164 bp	AF126041: 236 bp	AF143338: 267 bp	AF143357: 331 bp
AF143354: 164 bp	AF143340: 237 bp	AF143334: 271 bp	AF143350: 332 bp
AF126948: 183 bp	AF126039: 244 bp	AF143361: 271 bp	AF143355: 341 bp
AF125380: 184 bp	AF143347: 244 bp	AF113845: 275 bp	AF116185: 346 bp
AF143344: 185 bp	AF126040: 247 bp	AF143335: 276 bp	AF143339: 349 bp
AF143351: 187 bp	AF143343: 248 bp	AF143337: 281 bp	AF126950: 374 bp
AF127574: 190 bp	AF119787: 251 bp	AF143333: 282 bp	AF118927: 408 bp
AF143362: 194 bp	AF126946: 251 bp	AF116186: 284 bp	AF119788: 425 bp
AF118928: 199 bp	AF143367: 252 bp	AF118929: 291 bp	AF143353: 465 bp
AF125379: 209 bp	AF126044: 253 bp	AF143363: 305 bp	AF143352: 505 bp
AF126045: 210 bp	AF143349: 260 bp	AF126949: 311 bp	AF126044: 521 bp
AF143366: 220 bp	AF143360: 260 bp	AF143365: 323 bp	AF126947: 554 bp
AF125378: 225 bp	AF143356: 261 bp	AF127575: 324 bp	AF126042: 555 bp
AF143364: 226 bp	AF143358: 262 bp	AF136807: 327 bp	AF143342: 660 bp
AF143347: 230 bp	AF143359: 262 bp	AF143336: 327 bp	AF143346: 777 bp
AF143345: 232 bp			

Sequence data were registered at GenBank under the appropriate accession numbers as indicated beside the bp length of the fragments.

ing its inhibitor levamisole to the substrate solution. Nuclei were counterstained with Mayer's hemalaun solution (catalog no. 1.09249.0500; Merck, Darmstadt, Germany). Mounting of the slides was performed by the use of Kaiser's glycerin gelatin (catalog no. 1.09242.0100, Merck). Technical negative controls were performed by omitting the first antibody.

Results

The study identified more than 60 cDNA species that were expressed in the psoriatic plaque but not in the normal buccopharyngeal mucosa. When analyzing these cDNA data by comparison with GenBank data from the National Center for Biotechnology Information using the BLAST 2.0 search routine (www.ncbi.nlm.nih.gov/cgi-bin/BLAST), the main body of the cDNA species was found to contain until now unknown sequence information. The novel data were registered at GenBank receiving appropriate accession numbers and are communicated in Table 1.

A certain cDNA species with a 338-bp length, which appeared to be overexpressed in the psoriatic plaque as compared to oral mucosa as well as normal skin (Figure 1), was found to be identical with the hsRPB7 gene (GenBank identification U52427; corresponding positions 5553 to 5890) as a human homologue of yeast RPB7 coding the seventh subunit of RNA polymerase II.³¹⁻³³ Because of the until now unknown expression pattern of the hsRPB7 protein in psoriasis, we undertook a thorough immunohistological analysis giving the following results.

In peritonsillar mucosa there was no substantial expression of hsRPB7 detectable (Figure 2E). For psoriatic plaques we found a strong hsRPB7 expression mostly in the cytoplasm of the keratinocytes in the upper two thirds of the subcorneal epidermis, ie, in the stratum spinosum, whereas the basal and, in some instances, also the first suprabasal cell layers were spared (Figure 2, A-C). Normal epidermis and uninvolved psoriatic skin showed only a weak to moderate expression of hsRPB7 also restricted to the suprabasal layers (Figure 2D). Overall, we did not observe any substantial hsRPB7 expression in the capil-

Figure 1. A: Differential display gel demonstrating the psoriasis-related overexpression of a 338-bp cDNA species (arrow) that was eventually identified as representing the hsRPB7 gene coding a protein critically involved in DNA to mRNA transcription. S, bp-standard; M, peritonsillar mucosa; N, normal skin; P, psoriatic-involved skin. B: Sequence of the cDNA species containing part of exon 7, all of intron 7, and part of exon 8 from the hsRPB7 gene overexpressed in psoriasis plaque tissue as compared to peritonsillar oral mucosa.

Figure 2. Immunohistochemical detection of hSRPB7 protein expression as a red reaction product confined mostly to the cytoplasm of epidermal keratinocytes with blue counterstaining of the nuclei. In the psoriatic plaque the hSRPB7 protein was expressed strongly in the suprabasal and subcorneal epidermis with a sparing of the basal and, partly, the first suprabasal epidermal cell layers. Original magnifications: $\times 125$ (A), $\times 250$ (B), and $\times 500$ (C). In normal skin there was only a weak to moderate hSRPB7 expression [original magnification, $\times 500$ (D)], but again restricted to the suprabasal epidermal compartment without involvement of the basal epidermis. Notably, the peritonsillar mucosa showed only a faint hSRPB7 protein expression [original magnification, $\times 500$ (E)], confirming the RT/PCR differential display data. The cell differentiation-related tight down- and up-regulation of hSRPB7 as a critical transcription factor in the basal and suprabasal compartment, respectively, was proven as an obviously general phenomenon in normal (D) as well as psoriatic epidermis (C).

laries, inflammatory cells, or fibrocytic elements of the subepidermal or subepithelial compartments of involved and uninvolved psoriatic as well as normal skin nor peritonsillar mucosa, respectively.

To investigate the specificity of these findings for the psoriatic disease process, the immunohistological study was extended to some other forms of hyperkeratotic diseases. The hSRPB7 expression proved to be weak to moderate in seborrheic keratoses ($n = 3$) as well as in one case of ichthyosis vulgaris ($n = 1$), but to be very

strong in actinic keratoses ($n = 3$) representing precancerous lesions. Notably, in actinic keratoses the basal cell layers were not so strictly spared from the hSRPB7 expression.

Overall, a distinct pattern of RPB7 expression was observed, nearly completely missing in the peritonsillar mucosa epithelium, and being restricted to the suprabasal epidermis under nonpre-malignant conditions with a much higher intensity in involved psoriatic skin as compared to normal and uninvolved psoriatic skin.

Discussion

With regard to the contribution to the further elucidation of the etiopathogenesis of psoriasis, DD-RT/PCR techniques have already been used to compare involved and uninvolved psoriatic skin.³⁴ We now propose an alternate model comparing psoriatic plaque tissue and peritonsillar mucosa based on the clinical notion that despite the close microanatomical relation between both tissues, the latter is never involved by the psoriatic disease process. The DD/RT-PCR comparison of psoriatic-involved skin with normal peritonsillar mucosa is an attractive research objective aiming at the identification of gene products that might be critically involved in the etiopathogenesis of psoriasis with regard to 1) the epidermal hyperproliferation, 2) the vascular inflammatory compartment, as well as 3) putatively involved autoantigens. The project led to the discovery of so far unknown DNA sequence information that has to be further characterized and identified at the nucleotide and protein level.

The main outcome of our study is the hitherto unknown differential expression of the hsRPB7 gene in psoriatic and normal skin as well as peritonsillar mucosa. The corresponding product, which we picked up by DD-RT/PCR and identified by sequencing, contains part of exon 7, all of intron 7, and part of exon 8 from the hsRPB7 gene. Therefore this cDNA species may represent a pre-mRNA as well as an alternatively spliced form of hsRPB7 mRNA, in which intron 7 is not spliced out, leading to a modification in the C-terminus of the protein.

As a pivotal part of the DNA transcription machinery the hsRPB7 protein was found to be nearly absent in the peritonsillar mucosa epithelium and to be expressed suprabasally in the epidermis with a much higher abundance in involved psoriatic skin as compared to uninvolved psoriatic and normal skin. Overexpression of hsRPB7 did not seem to be strictly psoriasis-specific because we could observe this phenomenon also in actinic keratoses. However, there was a difference in the pattern of hsRPB7 expression between psoriatic plaques and actinic keratoses. Most interestingly, the latter expressed hsRPB7 also in major parts of the basal cell layer as a presumptive special feature of the disturbances related to the premalignant transformation occurring in these precancerous lesions.

Originally the RPB7 gene product has been characterized as a 19-kd subunit of the RNA polymerase II oligopolypeptide complex in the yeast *Saccharomyces cerevisiae* functioning as an essential factor for cell growth and viability.³⁵ Surface plasmon resonance studies demonstrated that the yeast RPB7 protein together with RPB4 stabilizes a pre-initiation complex consisting of promoter DNA, TATA box-binding protein, transcription factor TFIIB, as well as the RNA polymerase II being responsible for mRNA generation as a prerequisite for protein translation.³⁶

Only recently the existence of an evolutionarily conserved human homologue to RPB7 was shown, ie, hsRPB7.³¹⁻³³ Until now the knowledge about the regulation of mRNA and protein expression of hsRPB7 in the skin under physiological and pathophysiological condi-

tions is rather limited. Our data point to an up-regulation of hsRPB7 protein expression and thus DNA-mRNA transcription during the psoriasis dependently disturbed keratinocyte differentiation process in the upper epidermis. This finding seems to be another, novel facet reflecting the disturbed intraepidermal cell differentiation process in psoriasis. A very striking feature under psoriatic and normal tissue conditions was the spared hsRPB7 expression in the basal epidermal cells, an obvious cell differentiation-related phenomenon getting lost under premalignant conditions that awaits further characterization.

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