Effects of Expression of Transcriptional Factor AP-1 *FOSL1* Gene on Psoriatic Process

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We performed quantitative analysis of *FOSL1* gene expression in lesional psoriatic skin. The expression of this gene in lesional psoriatic skin was significantly increased compared to that in unaffected areas. Enhanced *FOSL1* expression significantly correlated with high psoriasis area and severity index (PASI). High level of *FOSL1* gene expression was proposed to be a marker of pathological process activity in psoriasis.

Key Words: *psoriasis; gene expression; real time polymerase chain reaction; FOSL1*

Psoriasis is a skin disease, which is a complex genetically determined pathology and involves large groups of interacting genes/proteins [5].

Transcriptome data processing showed that psoriasis determined by dysregulation of the genetic apparatus functioning is associated with enhanced transcription of numerous genes of transcriptional complex AP-1 [1,2]. Transcriptional factor AP-1 is a group of pairwise complexes formed by DNA-binding proteins of the Jun, Fos, and ATF (activating transcription factor) families [3].

A key position in this complex is occupied by *FOSL1* gene, which controls numerous cell functions. This gene is expressed in different types of cells with different intensity [12,13].

The role *FOSL1* as a proto-oncogene is now actively studied [4,5,8,10,11]. The main role of *FOSL1* gene is determined by its overexpression in various cell lines and clinical samples (epithelial cancer cells). Expression of this gene was found in various types of epithelial cells; enhanced *FOSL1* expression was also observed in fibroadenomas. Positive reaction for *FOSL1* was observed in some compartment of the bowel in the apical layer of stratified epithelium [14].

The aim of this study was to investigate changes in *FOSL1* expression in lesional psoriatic skin.

MATERIALS AND METHODS

Biopsy specimens of lesional psoriatic skin and unaffected skin from patients with psoriasis (10 patients) were taken under local anesthesia using dermatological punch. The samples were immediately frozen in liquid nitrogen, weighed, and grinded in a mortar, avoiding sample thawing.

RNA isolation from biopsy samples was performed on Qiagen columns according to standard RNeasy Mini Kit protocol.

Reverse transcription was carried out in 200 μl PCR test tubes. To this end, 5× M-MLV RT buffer, M-MLV reverse transcriptase (100 U, Promega), dNTP, RNase inhibitor RNasin (20 U, Promega), random hexanucleotide primers (Promega), and RNA up to a final concentration ≤100 ng/μl were added to the tube and the mixture was thermostated for 1 h at 37°C.

Electrophoresis was performed in horizontal agarose gel in TAE buffer (40 mM Tris-HCl, pH 8.0;

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1 mM EDTA; 20 mM CH_3COOH); 2% agarose gels were used.

Primers to *FOSL1* mRNA were chosen using Vector NTI Advance 10 software. Annealing temperature for each primer pair used in this study was chosen empirically, taking the temperature calculated in OligoCalculator software as the baseline. Human genomic DNA and total human cDNA were used as a the matrix during for choosing PCR conditions.

Real-time PCR was performed in 96-well optic plates using fluorescent-labeled oligonucleotide probes TaqMan®. The reaction was performed using 2.5× reaction mixture with referent stain ROX (Syntol). Primers and probes were synthesized by DNA-Synthesis company.

Amplification was performed in a PCR-amplifier (Bio-Rad, iQ4) according to following program: denaturation at 95°C for 4 min (stage 1), denaturation at 94°C for 30 sec (stage 2), and annealing and elongation at 60°C for 1 min (stage 3); stages 2-3 were repeated 50 times (stage 4). Genes and relevant primers with probes are presented (Table 1).

Expression of the target genes was standardized by the expression of housekeeping gene *GAPDH*.

For calculation of results the following parameters were used: PCR effectiveness at least 95%; correlation coefficient at least 0.99; slope -3.4±0.2.

Processing of PCR results was performed by $2^{-\Delta\Delta CT}$ method according to [7].

RESULTS

In previous bioinformatic investigations we used database GEO DataSets (http://www.ncbi.nlm.nih.gov/geo/), where the results of evaluation of gene expression levels on biochips are presented in electron tables.

MetaCore software (GeneGo Inc) was used as an instrument for processing of tabulated data. Processing of microarray data and the search psoriasis candidate genes were performed using this software product. The priority distribution of processes was carried out using Metacore software, assuming that lower p-value corresponds to higher relevancy of the genes. The data of bioinformatic analysis attested to the possible key role of *FOSL1* in the development of lesions and pathogenesis of psoriasis [1].

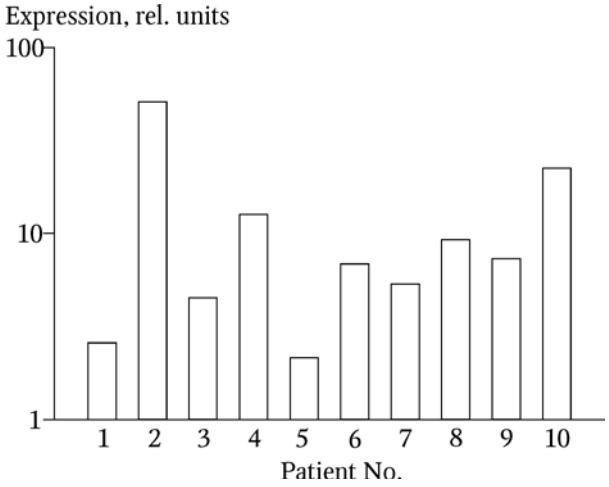

Fig. 1. Changes in *FOSL1* expression level in lesional psoriatic skin.

The levels of *FOSL1* expression in lesional psoriatic skin were compared with those in unaffected skin using Real Time PCR.

Patient state was assessed using psoriasis area and severity index (PASI). Local PASI was estimated for each part of the body. Integral PASI was equal to the sum of local indices and could vary from 0 to 72 units. The maximum and minimum PASI values in the given group were 11.4 and 1.8, respectively (Table 2).

The experiment showed that *FOSL1* expression in lesional psoriatic skin in virtually all patients was 2-fold higher than in unaffected skin. In patients 2, 4, 10, *FOSL1* expression was almost 10-fold higher: 51.0321, 12.6699, and 22.3675, respectively (Fig. 1). It should be noted that in patient with the highest PASI values gene expression increased more than 10-fold; high *FOSL1* expression levels corresponded to higher psoriasis severity.

The data on the relationship between *FOSL1* and psoriasis are contradictory. Some investigators reported decreased expression of this gene in the skin of patients with psoriasis [6], while in other studies, changes in the expression of other members of AP-1 family, but not *FOSL1* were observed [15]. Apparently, further investigations of *FOSL1* as a gene participating in psoriasis development are needed.

TABLE 1. Primers and Probes Used for Evaluation of Gene Expression Using Real-Time PCR

No.	Name	Sequence 5'-3'	PCR product length, b.p.s
1	*GAPDH*	Primer kit and probe were obtained from DNA-Synthesis	200
3	*FOSL1*	**FAM**-CGCGATCAGAAGAAGTTGCTGGAGTTGGATGTGGGATCGCG-**BHQ1**	
		CACCCTAGCCAATGTCTCC	
		GAAGCACAATATGGTCAGTCTC	120

TABLE 2. Clinical Parameters of Patients with Psoriasis

Patient No.	Changes in PASI values	Regression of subjective signs: itch, pain, tension (4-point scale)
1	4.0	1
2	7.2	2
3	1.8	2
4	9.4	3
5	2.1	2
6	4.2	0
7	2.1	2
8	2.8	0.6
9	3.2	0
10	11.4	2.0

Generally, enhanced *FOSL1* expression in all examined patients in our study attests to possible key role of *FOSL1* gene in psoriasis development.

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