Comparative Study of the Expression of *ATF-3* and *ATF-4* Genes in Vessels Involved into Atherosclerosis Process and in Psoriatic Skin

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Expression of *ATF-3* and *ATF-4* genes was examined quantitatively by real-time PCR and changes in the expression of these genes in atherosclerotic lesions and in psoriatic skin were demonstrated. It was found that concomitant pathologies do not affect the expression of these genes. Opposite changes in the expression of *ATF-3* and *ATF-4* genes in atherosclerotic and psoriatic samples were revealed and a hypothesis was put forward that this parameter could be a criterion of pathological process in both diseases.

Key Words: *atherosclerosis; psoriasis; gene expression; real-time PCR; transcription complex AP-1*

Activating transcription factor (ATF) is a component forming transcription complex activator protein-1 (AP-1) involved in the regulation of a number of key processes (morphogenetic cell proliferation, differentiation, transformation, and apoptosis) in response to a number of extracellular factors and various signaling molecules, including growth and paracrine factors, cytokines, oncogenes, tumor promoters, chemical carcinogens, peptide hormones, and neurotransmitters [11].

Factors of the ATF family have been identified as cell proteins binding to certain sites in the promoter of some adenovirus early genes. ATF DNA-binding site is identical to that of transcription factor CREB (cAMP responsive element binding protein 1). Primary structures of ATF and CREB are also similar. Factors of the ATF/CREB family can be divided into six main groups: CREB (CREM and ATF-1 are also included), ATF-2 (CREBP-1 is included), ATF-3, ATF-4, ATF-6, B-ATF. All these proteins form dimeric complexes [4,8,14].

The involvement of ATF gene as components of the transcription complex AP-1 family in the pathogenesis of such social diseases as Crohn's disease and psoriasis has been previously demonstrated by bioinformatic analysis [1,2].

Since the formation of psoriatic plaques on the skin and atherosclerotic plaques in cardiovascular diseases has been found to be mediated by similar molecular mechanisms and a relationship between lipid metabolism and the parameters plasma cholesterol in the epidermis has been demonstrated [3,5-7,9], we studied the role of ATF gene family in atherosclerosis and psoriasis.

MATERIALS AND METHODS

Atherosclerosis autopsy material (n=16) was collected from the abdominal aorta with signs of atherosclero-

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sis: atheromatous plaques (n=8) and visually normal part of the vessel at the prelipid stage of atherogenesis (n=8). The samples were taken with a lancet by separating the intima from the media, washed in 0.9% NaCl, and frozen in liquid nitrogen.

Skin biopsy specimens from patients with *Psoriasis vulgaris* were taken under local anesthesia from lesions (all were plaque-type) and normal skin sites with dermatological 4-mm punch. The patients received no systemic or PUVA/UV therapy within 1 month before skin biopsy. Normal skin samples were taken at least 3 cm distant from psoriatic lesions (a total of 20 specimens: 10 from psoriatic lesions and 10 from visually normal skin). The study was approved by local ethics committee of the Institute of General Genetics and complies with the principles of the Helsinki Declaration.

RNA from biopsy samples was isolated 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 to a final concentration of ≤100 ng/µl were added to the tube and the mixture was incubated for 1 h at 37°C.

Primers to *ATF-3* and *ATF-4* mRNA were designed using Vector NTI Advance 10 software. The primer annealing temperature was selected experimentally; the initial temperature was calculated using OligoCalculator software.

Real-time PCR was performed in 96-well optical plates using 2.5× reaction mixture with the ROX reference dye (Sintol). The primers and probes were synthesized by DNK Sintez

Amplification was carried out in an iQ4 thermal cycler (Bio Rad) using the following two step program: stage 1: denaturation at 95°C for 4 min; stage 2: denaturation at 94°C for 30 sec; stage 3: annealing and elongation at 60°C for 1 min; stage 4: stage 2 and stage 3 repeated 50 times. Otherwise, the following three step program was used: stage 1: denaturation at 95°C for 4 min; stage 2: denaturation at 94°C for 15 sec; stage 3: annealing at 55°C for 15 sec; stage 4: elongation at 72°C for 15 sec; stage 5: stages 2-4 repeated 45 times.

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

A series of four tenfold dilutions depending on the concentration of the sample was used as standards.

The results were processed assuming the parameters of PCR reactions as follows: reaction efficiency >95%, correlation coefficient ≥0.99, and slope -3.4±0.2.

Method $2^{-\Delta\Delta Ct}$ was used to analyze PCR results [7].

RESULTS

On the basis of the results of bioinformatic analysis [1,2] we hypothesized that protein components of the transcription factor AP-1 may play a key role in the formation of psoriatic and atherosclerotic plaques and compared the expression of genes *ATF-3* and *ATF-4* in atherosclerotic lesions and normal vessels and in psoriatic lesions and normal skin.

Autopsy samples were collected from patients treated for different diseases, primarily related to circulatory disorders. The main diagnoses were ischemic stroke, small-focal cardiosclerosis, and macrofocal postinfarction cardiosclerosis. Exertion angina, postinfarction cardiosclerosis, and consequences of cerebral infarction (brown cysts in the cores of the cerebral hemispheres) were most prevalent comorbidities.

Other etiologies not related to circulatory disorders were also diagnosed (breast cancer with tumor degeneration and metastases to regional lymph nodes and left hemisphere of the brain, gastroduodenal ulcer).

Examination of psoriatic and visually uninvolved skin specimens was conducted in a group of 10 patients with psoriasis. Psoriasis area and severity index (PASI) was used for evaluation of the severity of psoriasis. The maximum and minimum PASI values in this group were 11.4 and 1.8, respectively (Table 1).

The expression of *ATF-3* gene in atherosclerotic plaques differed from that in normal vessels and in affected psoriatic skin differed from that in normal skin (Fig. 1).

In all the patients, expression of *ATF-3* gene in atherosclerotic vessels increased by more than 2 times

TABLE 1. Clinical Parameters of Patients with Psoriasis

Patient number	PASI changes	Regression of subjective symptoms: itching, pain, feeling of tension (score by a 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

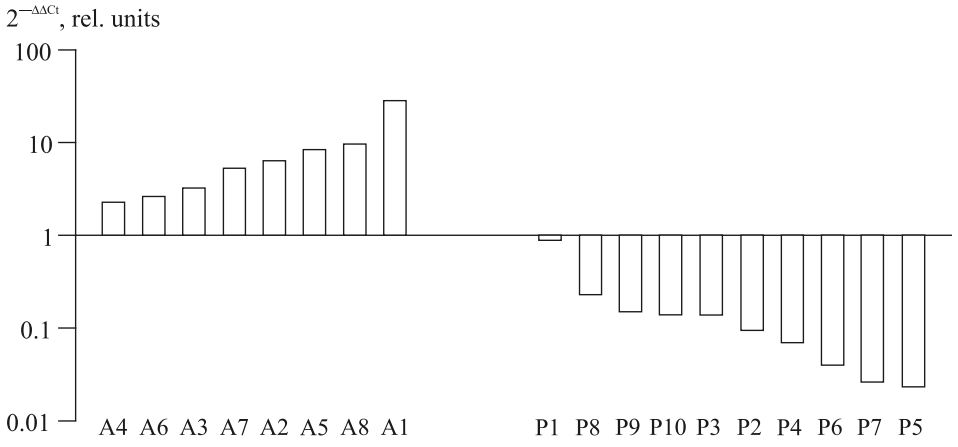

Fig. 1. Changes in the expression of *ATF-3* gene in atherosclerotic plaques compared to visually unaffected vessels (A1-A8) and in psoriatic skin compared to the visually normal skin (P1-P10).

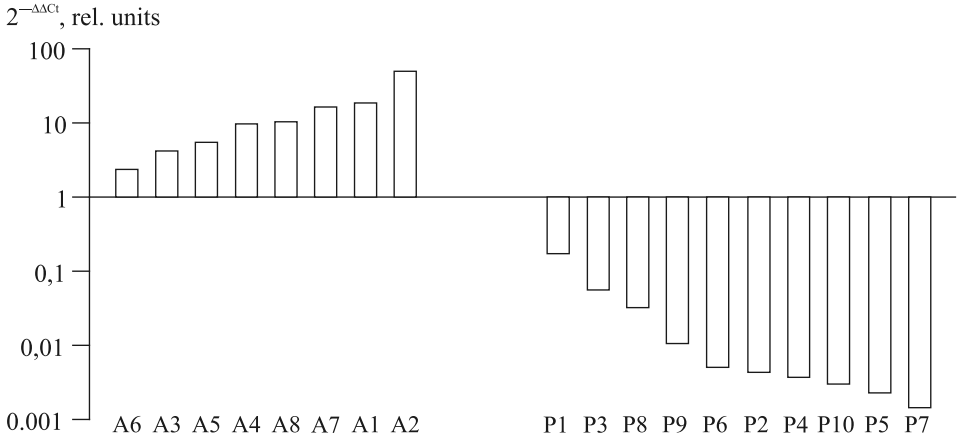

Fig. 2. Changes in the expression of *ATF-4* gene in atherosclerotic plaques compared to visually unaffected vessels (A1-A8) and in psoriatic skin compared to the visually normal skin (P1-P10).

compared to visually unaffected vessels and in affected psoriatic skin this parameter decreased by more than 2 times compared to visually unaffected skin. Changes in the expression of *ATF-4* gene were similar (Fig. 2).

It should be noted that the comorbidity found in patients with atherosclerosis does not affect changes in the expression of the studied genes.

The expression of *ATF-3* and *ATF-4* genes in psoriatic skin lesions did not depend on the severity of the process (index PASI).

Thus, the results attest to dysregulation and opposite changes in the expression of *ATF-3* and *ATF-4* genes in atherosclerosis and psoriasis: gene expression was enhanced in all specimens of atherosclerosis plaques and reduced in all psoriatic lesions.

ATF-3 is an immediate early response gene in vascular endothelial cells and can play an important role in endothelial cell death associated with atherogenesis [8]. It is known that death of vascular endothelial cells contributes to the progression of the atherosclerotic lesions. Involvement of *ATF-4* in the induction of VEGF and E-selectin may be related to the development of inflammation. In addition, ATF-4 is required for the induction of transcription activating factor 3 (ATF-3) [10].

It is known that ATF-3 and ATF-4 form dimeric complexes with c-Jun, another subunit of AP-1 transcription factor [4]. In turn, c-Jun is a negative regulator of the psoriatic process [15]. Hence, the expression of these genes should be strongly reduced in psoriatic skin, which is confirmed by our results.

Opposite changes in the expression of *ATF-3* and *ATF-4* genes in atherosclerotic and psoriatic specimens suggest that this parameter could be a criterion of pathological process in both diseases.

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