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Microarray Analysis of NF-κB-dependent Genes in Chronic Rhinosinusitis with Nasal Polyps*

Analiza profilu ekspresji genów zależnych od czynnika transkrypcyjnego NF-κB w przewlekłym zapaleniu zatok przynosowych z polipami

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. The inflammatory process underlying nasal polyposis is induced and perpetuated by the enhanced activity of several agents including transcription factors. It has recently been demonstrated that one of them, named nuclear factor-kappa B (NF-κB), is implicated in the regulation of multiple pro-inflammatory genes.
Objectives. The aim of the study was to identify using microarray technology which NF-κB-dependent genes are activated in nasal polyp (NP) samples compared to the control mucosa.
Material and Methods. The transcriptional activity of genes was analyzed using an oligonucleotide microarray on 15 NPs and 8 cases of normal nasal mucosa.
Results. Gene expression patterns obtained in NPs were significantly different from those in normal mucosa. NPs and control cases clustered separately, each of them with large homogeneity in gene expression. Among 582 human NF-κB-dependent genes 25 showed a significantly higher expression in NPs compared to the control. The largest increase focused on gene encoding *TFF3* (a 5-fold higher expression) followed by *NOS2A* (5x), *SERPINA1* (4x), *UCP2* (4x), *OXTR* (4x) and *IL8* (3x) ($p < 0.05$). In healthy mucosa 19 genes presented increased transcription activity compared to NPs. The most significantly enhanced levels were shown in case of *LTF* gene (20 fold) followed by *KRT6B* (7x), *LYZ* (7x), *SD11B2* (5x) and *MMP3* (4x) ($p < 0.05$).
Conclusions. DNA microarray technology highlights the involvement of many unsuspected pathologic pathways which could be involved in NP growth. The identification of novel disease-related genes may help to understand the biology of NPs and elaborate new targeted therapy (**Adv Clin Exp Med 2013, 22, 2, 209–217**).

Key words: chronic rhinosinusitis, nasal polyps, DNA microarray, NF-κB.

Streszczenie

Wprowadzenie. W złożonej etiologii przewlekłego zapalenia zatok przynosowych z polipami (PZZPzP) istotną rolę pełni zwiększona aktywność niektórych czynników transkrypcyjnych. Jednym z nich jest czynnik transkrypcji jądrowej NF kappa B (NF-κB), który reguluje ekspresję wielu genów kodujących białka o właściwościach prozapalnych.
Cel pracy. Ocena profilu ekspresji genów zależnych od aktywności NF-κB w PZZPzP w porównaniu z niezmienioną błoną śluzową jamy nosa osób z wykluczonym procesem zapalnym.

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Materiał i metody. Aktywność transkrypcyjna genów została oceniona metodą mikromacierzy oligonukleotydowych w grupie 15 pacjentów z PZZPzP i w 8-osobowej grupie kontrolnej.
Wyniki. Profil ekspresji genów w PZZPzP różnił się istotnie w porównaniu z profilem w niezmienionej błonie śluzowej jamy nosa. Analiza skupień wyróżniła dwie oddzielne grupy pacjentów (z PZZPzP oraz grupę kontrolną). Obie charakteryzowały się dużą jednorodnością ekspresji genów. Spośród 582 genów zależnych od czynnika transkrypcyjnego NF-κB 25 miało istotnie zwiększoną ekspresję w PZZPzP w porównaniu z grupą kontrolną. Były to geny kodujące białka: TFF3 (5x większa ekspresja), NOS2A (5x), SERPINA1 (4x), UCP2 (4x), OXTR (4x) i IL8 (3x) ($p < 0.05$). W grupie kontrolnej 19 genów charakteryzowała zwiększona ekspresja w stosunku do grup z PZZPzP. Największą różnicę ekspresji wykazały geny: *LTF* (20x większa ekspresja), *KRT6B* (7x), *LYZ* (7x), *SD11B2* (5x) i *MMP3* (4x) ($p < 0.05$).
Wnioski. Analizowanie profilu ekspresji genów z użyciem mikromacierzy oligonukleotydowych sugeruje istnienie niezbadanych dotąd szlaków patologicznych, które mogą mieć znaczenie w etiologii PZZPzP. Identyfikacja nowych genów związanych z PZZP może pomóc w zrozumieniu biologii tego schorzenia, a w przyszłości opracowaniu nowej celowanej terapii (**Adv Clin Exp Med 2013, 22, 2, 209–217**).

Słowa kluczowe: zapalenie zatok przynosowych, polipy nosa, mikromacierze DNA, NF-κB.

During chronic inflammation within paranasal sinuses the growth of nasal polyps (NPs) is induced and perpetuated by the complex interaction of various cytokines produced by structural and infiltrating cells. It is known that chemokines expression during that process is inspired by several transcription factors. It has recently been demonstrated that one of them, named nuclear factor-kappa B (NF-κB), is implicated in the regulation of multiple pro-inflammatory genes likely to participate in the etiology of chronic rhinosinusitis.

The inactive form of NF-κB resides in the cytoplasm and is constituted by a heteromeric complex of p50, p65 and an inhibitor of the NF-κB (IκB) family of proteins. Activation of NF-κB, in response to a variety of extracellular signals, leads to degradation of IκB. Released p50-p65 heterodimer translocates to the nucleus where it binds to specific consensus sequences within the promoter of NF-κB target genes [1]. NF-κB is activated at sites of inflammation in different diseases where it is implicated in the regulation of more than 500 genes involved in important biological processes [2].

Takeno et al. [3] reported a correlation between an increase in p50 subunit level and the expression of IL-8, IL-16 and eotaxin which suggests that NF-κB may have an important role in the perpetuation of the inflammatory process in NPs. NF-κB acts as anti-apoptotic molecules in eosinophils and may be responsible for the recruitment and enhanced survival of inflammatory cells. Moreover, it was observed that mice deficient in the p50 have shown a lack of eosinophilic accumulation in the airway inflammation compared with wild-type animals [4].

Since NF-κB coordinates and amplifies the immune and inflammatory response, selective inhibition of its activity could be useful, either as single agents or associated with conventional therapy. Several reports have pointed to NF-κB as an interesting new therapy targeting the inactivation which leads to tumor cell death or growth inhibition [5].

The aim of the present study was to indicate using microarray technology which NF-κB-dependent genes are activated in NP samples compared to the control mucosa.

Material and Methods

Subjects

Fifteen patients (9 males and 6 females; mean age 51.23 ± 18.21) with NP treated surgically at the Department of Otolaryngology, Wroclaw Medical University were included into the studied group according to diagnostic criteria for chronic rhinosinusitis established by the Task Force on Rhinosinusitis (AAO-HNS). Patients had been free of any medication for at least 4 weeks before surgery and had bilateral polyps in the nasal cavities. Patients with an established immunodeficiency, allergic fungal sinusitis, ciliary dyskinesias, sinonasal tumor, bronchial asthma, pollen allergy or cystic fibrosis were excluded from the study.

The control group included 8 healthy individuals (5 males and 3 females; mean age 39.4 ± 11.2). All control tissue samples were taken from unchanged nasal mucosa of uncinate process during nasal trauma surgery or DCRs. The absence of NP was assessed through clinical history, endoscopic examination and imaging. Negative prick tests and an absence of allergies in the personal history were required. Other diseases were also excluded.

Tissue samples were flash frozen in liquid nitrogen immediately after harvesting and stored at –70°C before RNA extraction. A portion of each sample was routinely elaborated for subsequent immunohistochemical examination to visualize inflammatory cells and to exclude other pathologies. The presence of edema or inflammation was ruled out in healthy mucosa. The diagnosis of eosinophilic NP was determined if the percentage of eosinophils was greater than 80% of all leukocytes or the presence of clusters of eosinophils was

noted. When the dominant cells in the tissue were lymphocytes and plasmocytes, the diagnosis of non-eosinophilic NP was established. On the basis of that criterion 9 (60%) cases were classified as eosinophilic NP and 6 (40%) as non-eosinophilic ones.

Ethical Considerations

The study was approved by the Local Ethics Committee of Wroclaw Medical University. All patients were informed about the research and signed an informed consent form.

RNA Extraction

Total RNA was extracted from frozen tissue specimens (about 40 mg) with the use of TRIzol® reagent (Invitrogen, USA) according to the producer's protocol. Tissue samples were homogenized in 1 ml TRIZOL® reagent using the Polytron (Kinematica AG, Switzerland) tissue homogenizer. All RNA extracts were treated with DNA-se I and cleaned by Mini-spin column using an Rneasy Mini Total RNA Purification kit (Qiagen, USA).

The RNA extracts were qualitatively checked by electrophoresis in 1.0% agarose gel stained with ethidium bromide. RNA concentration was determined spectrophotometrically using a GeneQuant pro (Biochrom, UK). RNA purity was judged by the ratio of absorbance at 260 and 280 nm (A_{260}/A_{280}) (ratios between 1.9 and 2.1 were acceptable). An RNA integrity test was performed using agarose gel electrophoresis and staining the RNA with ethidium bromide.

Microarray Procedure

Gene expression profiles were determined using commercially available oligonucleotide microarrays HG-U133A (Affymetrix, USA) as described previously [6]. Each gene chip contains 22 238 probe sets that correspond to more than 18 400 transcripts and 14 500 well-characterized human genes. Briefly, 8 μg of total RNA from every sample were used for the cDNA synthesis using SuperScript Choice System (Gibco BRL Life Technologies, UK). In the next step cDNA were templated to generate biotin-labeled cRNA using a BioArray HighYield RNA Transcript Labeling Kit (Enzo Life Sciences, USA). cRNA was purified on Rneasy Mini Kit columns (Qiagen). Next, 16 μg biotin-labeled cRNA was fragmented by using a Fragmentation Buffer (Qiagen) at 94°C before hybridization to gene chips for 16h at 45°C. The hybridized cRNA probe was stained with streptavidin-phycoerythrin conjugate and scanned using a G2500A GeneArray Scanner.

Microarray Data Analysis

The resulting images were analyzed using Microarray Suite 5.0 software (Affymetrix). Results were normalized with RMAExpress version 0.5 Release (http://rmaexpress.bmbolstad.com). Statistical analysis was performed with the use of Statistica 6.0 software (StatSoft, Poland). Cluster analysis (Ward's method) was applied to the normalized data. To analyze the microarray data the t test for two unpaired groups was used. The criterion for estimating the differentially expressed genes was fold change (FC) of ≥ 2 or greater. Fold change is calculated from the signal log ratio (SLR) (values following log2 used as the ratio between 2 groups). Genes are up-regulated if SLR ≥ 0 and $FC = 2^{SLR}$ and down-regulated by SLR < 0 and $FC = 2^{-(SLR),}$ respectively. Genes were considered as potentially differencing if $p < 0.05$ and there was at least a 2-fold change in the mean expression level between NP and the controls.

Results

In this study oligonucleotide microarrays were used to investigate the expression of human NF-κB-dependent genes in NP tissues and normal healthy mucosa. On the basis of the Affymetrix database (http://www.affymetrix.com/analysis/index.affx) 582 genes controlled by NF-κB transcription factor were chosen.

First, hierarchical clustering analysis was performed to find similarities in gene expression profiles. Grouping based on NF-κB-dependent gene expression fingerprints revealed two distinct groups. NP patients clustered together indicated large homogeneity in gene expression. Similarly, healthy mucosa cases presented a consistent gene expression profile but different to NPs (Fig. 1). Analysis of NF-κB-dependent gene using oligonucleotide microarrays failed to distinguish eosinofilic from non-eosinophilic nasal polyps.

In the next stage of analysis the authors identified genes showing differential expression in NPs and normal nasal mucosa (Fig. 2). Among 582 human NF-κB-dependent genes, 25 showed a significantly higher expression in NPs compared to the control (Tabl. 1). The majority of the genes showed expression levels from 2 to 3-fold (19 genes) higher than in healthy mucosa. The largest increase was revealed in case of gene encoding *TFF3* (5-fold higher expression) followed by *NOS2A* (5x), *SERPINA1* (4x), *UCP2* (4x), *OXTR* (4x) and *IL8* (3x).

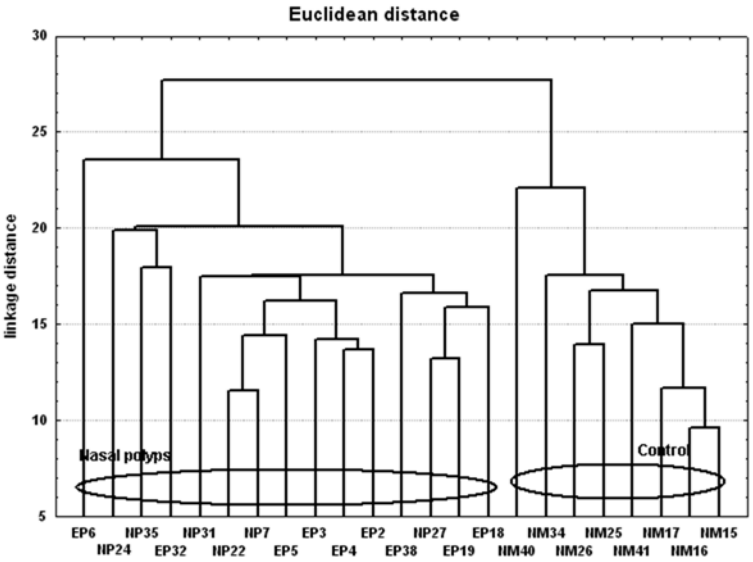

Fig. 1. Dendrogram of 15 nasal polyps (eosinophilic polyps (EP), non-eosinophilic polyps (NP)) and 8 normal mucosa (NM) samples using cluster analysis based on the Euclidean distance with 582 human NF-κB-dependent genes represented on the HG-U133A GeneChip

Ryc. 1. Dendrogram przedstawiający wynik analizy skupień 15 pacjentów z polipami nosa (polipy eozynofilowe (EP), polipy nieoeozynofilowe (NP)) oraz 8 wycinków niezmienionej błony śluzowej jamy nosa (NM) na podstawie odległości euklidesowej po ocenie ekspresji 582 genów zależnych od czynnika transkrypcyjnego NF-κB. Analizę wykonano techniką mikromacierzy oligonukleotydowych z użyciem matrycy HG-U133A

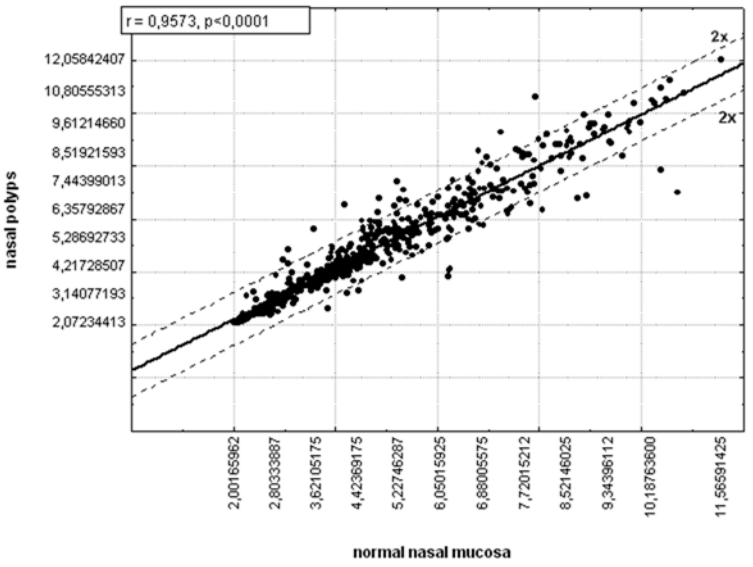

Fig. 2. Scatter plots of gene expression in nasal polyps and normal tissues (x-axis represents the normalized signal value of normal nasal mucosa, and y-axis represents the normalized signal value of NP)

Ryc. 2. Wykres punktowy ekspresji genów w tkankach polipów nosa i niezmienionej zapalnie błonie śluzowej jamy nosa

In healthy mucosa 19 genes presented increased transcription activity compared to NPs (Table 2). Most of the genes (11 genes) revealed only a 2 to 3-fold difference in expression. The most significantly enhanced level was shown in case of *LTF* gene (20 fold) followed by *KRT6B* (7x), *LYZ* (7x), *SD11B2* (5x) and *MMP3* (4x).

Discussion

In the present study DNA microarray technology was applied to identify differential gene expression in NP tissues and in healthy nasal mucosa. DNA microarray consists of a matrix with attached sequences that allow analysis of the expression of whole panels of genes and indicate those which have been activated in the disease state.

Active fraction of NF-κB induces transcription of genes including *IL-8*, *IL-16*, *iNOS*, and eotaxin which upregulation has been previously implicated in the pathogenesis of NPs [4]. Higher transcriptional activity of these genes was also observed in our study. *CCL11* (eotaxin 1) showed an almost 3 fold higher expression in NPs compared to the control. Eotaxins are the most potent chemoattractant for eosinophils, and are thought to play a key role in tissue eosinophilia. In such a mechanism eotaxins increase local inflammatory status and are strongly involved in the development of NPs [7]. IL-8 was the most significantly up-regulated NF-κB dependent interleukin in our study. Locally produced IL-8 contributes to the progression of chronic inflammation in the sinus mucosa. It is postulated that IL-8 participates in the recruitment and activation of leukocytes, T lymphocytes and eosinophils [8]. However, when challenged with

Table 1. The list of NF-κB-dependent gene showing higher expression in nasal polyps compared to normal mucosa identified by oligonuclotide microarray. * t test

Tabela 1. Lista genów zależnych od czynnika transkrypcji jądrowej NF-κB charakteryzujących się wyższą ekspresją w tkankach polipów nosa niż w grupie kontrolnej. * t test

Probe set (Zestaw próbek)	Gene symbol (Symbol genu)	Gene name (Nazwa genu)	Fold change (Krotność zmiany)	p*
204623_at	TFF3	trefoil factor 3	6.74	p < 0.0001
210037_s_at	NOS2A	nitric oxide synthase 2A	5.23	p = 0.0002
211429_s_at	SERPINA1	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase antitrypsin), member 1	4.71	p < 0.0001
208998_at	UCP2	uncoupling protein 2	4.16	p < 0.0001
202859_x_at	IL8	interleukin 8	3.88	p = 0.0014
206825_at	OXTR	oxytocin receptor	3.55	p = 0.0008
204470_at	CXCL1	chemokine (C-X-C motif) ligand 1	3.51	p = 0.0013
209875_s_at	SPP1	Secreted phosphoprotein 1	2.86	p = 0.0025
201859_at	PRG1	p53-responsive gene 1	2.78	p = 0.0002
210133_at	CCL11	chemokine (C-C motif) ligand 11; eotaxin-1	2.74	p = 0.0056
201645_at	TNC	Tenascin C (hexabrachion)	2.66	p < 0.0001
208451_s_at	C4A	complement component 4A	2.57	p = 0.0018
201042_at	TGM2	transglutaminase 2	2.56	p = 0.0002
202357_s_at	CFB	complement factor B	2.5	p = 0.0194
214211_at	FTH1	ferritin, heavy polypeptide 1	2.4	p < 0.0001
204475_at	MMP1	matrix metallopeptidase 1	2.39	p = 0.0223
204673_at	MUC2	mucin 2, oligomeric mucus/gel-forming	2.39	p = 0.0404
201998_at	ST6GAL1	ST6 beta-galactosamide alpha-2,6-sialyltranferase 1	2.29	p = 0.0002
204655_at	CCL5	chemokine (C-C motif) ligand 5	2.17	p = 0.0009
201313_at	ENO2	enolase 2 (gamma, neuronal	2.16	p < 0.0001
203828_s_at	IL32	interleukin 32	2.11	p = 0.0052
202718_at	IGFBP2	insulin-like growth factor binding protein 2	2.08	p < 0.0001
207076_s_at	ASS1	argininosuccinate synthetase 1	2.06	p = 0.0005
201510_at	ELF3	E74-like factor 3 (ets domain transcription factor, epithelial-specific)	2,06	p = 0.0186
205692_s_at	CD38	CD38 molecule	2.05	p = 0.0006
214428_x_at	C4B	complement component 4B	2.03	p = 0.0024
217767_at	C3	complement component 3	2.03	p = 0.0417
203936_s_at	MMP9	matrix metallopeptidase 9	2.0	p = 0.0362
210136_at	MBP	myelin basic protein	2.0	p = 0.0475
202404_s_at	COL1A2	collagen, type I, alpha 2	2.0	p = 0.0470

Table 2. The list of NF-κB-dependent gene showing higher expression in normal mucosa than in nasal polyps identified by oligonuclotide microarray. * t test

Tabela 2. Lista genów zależnych od czynnika NF-κB, których ekspresja była większa w grupie kontrolnej w porównaniu z polipami nosa. * t test

Probe set (Zestaw próbek)	Gene symbol (Symbol genu)	Gene name (Nazwa genu)	Fold change (Krotność zmiany)	p*
202018_s_at	LTF	lactoferrin	12.78	p = 0.0003
209126_x_at	KRT6B	keratin 6B	5.8	p = 0.0009
205828_at	MMP3	matrix metallopeptidase 3	5.05	p < 0.0001
204130_at	HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2	4.3	p < 0.0001
219403_s_at	HPSE	heparanase	4.23	p < 0.0001
212657_s_at	IL1RN	interleukin 1 receptor antagonist	4.08	p = 0.0016
213975_s_at	LYZ	lysozyme	3.91	p = 0.0030
209189_at	c-FOS	FBJ murine osteosarcoma viral oncogene homolog	3.22	p = 0.0420
207206_s_at	ALOX12	arachidonate 12-lipoxygenase	2.81	p < 0.0001
205392_s_at	CCL14	chemokine (C-C motif) ligand 14	2.28	p = 0.0003
205440_s_at	NPY1R	neuropeptide Y receptor Y1	2.26	p = 0.0002
204734_at	KRT15	keratin 15	2.25	p = 0.0225
202431_s_at	MYC	v-myc myelocytomatosis viral oncogene homolog	2.21	p = 0.0024
220987_s_at	SNARK	SNF1/AMP activated protein kinase	2.16	p < 0.0001
209396_s_at	CHI3L1	chitinase 3-like 1 (cartilage glycoprotein-39)	2.15	p < 0.0001
201694_s_at	EGR1	early growth response 1	2.05	p = 0.0354
202555_s_at	MYLK	myosin light chain kinase	2.0	p = 0.0072
202936_s_at	SOX9	SRY (sex determining region Y)-box 9	2.0	p = 0.0004
207356_at	DEFB4	defensin, beta 4	2.0	p = 0.0372
203234_at	UPP1	uridine phosphorylase 1	2.0	p = 0.0397
204621_s_at	NR4A2	nuclear receptor subfamily 4, group A, member 2	2.0	p = 0.0002

IL-8 of primed nasal mucosa a significant influx of neutrophils but not eosinophils was induced [9]. In airway epithelium, IL-8 is one activator of MMP-9 and MMP-8 expression and secretion during airway remodeling [10]. IL-8 and MMP-8 may form a pivotal inductive cytokine-proteinase cascade in the pathogenesis of NP.

The next gene known for its involvement in NP growth showing enhanced transcriptional activity in the present study encodes nitric oxide synthase (NOS). NOS is the main source of nitric oxide (NO) in the respiratory tract. It was revealed that NO may cause microvascular leakage and stimulation of glandular secretions contributing to mucosal edema formation characteristic for NPs [11]. Subsequent vascular dilatation in the nasal mucosa causes increased nasal airway resistance, which could facilitate the local release and generation of mediators induced by inflammatory cells [12].

The expression of the majority of genes presenting higher transcriptional activity in the present study has not been widely investigated in NP to date. Among all the examined genes, *TFF3* (trefoil factor 3) was the most significantly expressed in NPs compared to the control. TFF3 is mainly synthesized by mucin-secreting epithelial cells and participate in mucosal surface protection and repair after injury [13]. Since TFF3 inhibits apoptosis, it demonstrates prosurvival as well as proinvasive and proangiogenic activities [14]. It was found that IL-4 and IL-13 involved in NP growth induce TFF3 expression via a direct effect on epithelial cells [15]. In oral keratinocytes stimulated with TFF3 increased transcription of

genes related to cell survival, cellular growth and proliferation was showed [16].

SERPINA1 (alpha1-antitrypsin, AAT) activity has previously been found to be elevated in chronic sinusitis which was presumed to reflect its protective function, i.e. neutralization of neutrophil elastases which can be a driving force of mucosal inflammation [17]. SERPINA1, an acute phase protein, is one of the major serine protease inhibitors in the human body. AAT has been shown to increase fibroblast proliferation, to inhibit neutrophil chemotaxis, superoxide production and cell apoptosis [18]. AAT specifically enhanced LPS induced RANTES expression in BAL and IL-10, IL-12 and IL-13 levels in lung homogenates [19]. All of this indicates that SERPINA1 plays an important regulatory role in various inflammatory cascades.

UCP2 (uncoupling protein 2) is another gene which exhibits a distinctly higher expression in NP samples. UCP2 belongs to a newly discovered subgroup of mitochondrial carrier proteins. Its precise function is still unclear. UCPs regulate production of reactive oxygen species (ROS), inhibit inflammation as well as cell death [20]. UCP2 is expressed in many cells including infiltrating immune cells like macrophages, lymphocytes, dendritic cells and neutrophils. Since ROS modulates activation of T and B lymphocytes, UCP2 may influence the function of these cells [21]. As a regulator of mast cell UCP2 has potential implications for treatment of mast cell-mediated allergic and inflammatory diseases. It has been proved that UCP2 negatively regulates mast cell degranulation, histamine production, as well as IL-6 and PGD2 and release [22]. Thus, inhibition of UCP2 may rather worsen allergic and inflammatory diseases while its activation may act to reduce inflammatory responses.

Similar to the IL-8, CXCL1 is of particular interest as a chemoattractant and activator of neutrophils. This growth-related oncogene is crucial for the recruitment of other inflammatory cells including monocytes, dendric and T cells. Both transcripts and protein levels for CXCL1 were noted to be significantly elevated in NP and also released by infiltrating eosinophils [23].

Besides IL-8, the authors revealed another upregulated interleukin in NP tissues. IL-32 is a recently described pro-inflammatory cytokine produced mainly by epithelial cells, T lymphocytes, natural killer cells and monocytes [24]. IL-32 induces the expression of other important pro-inflammatory cytokines (IL-1β, IL-6, TNF-α and IL-8) and may further amplify inflammatory reaction [24].

Secreted phosphoprotein 1 (SPP1; also known as early T lymphocyte activation protein 1) is a phosphorylated glycoprotein classified as a Th1 cytokine involved in the inflammatory and immunological processes. SSP1 has strong pro-inflammatory properties and plays an important role in recruiting inflammatory cells and regulating the function of monocyte, macrophage, dendritic cells, Th1 and B cells. Eosinophils are an important source of SSP1 production in NP which implies a role for that cytokine in the etiology of NPs [25]. In sinonasal mucosa SSP1 could induce production of cytokines strictly involved in NP development like IFN-γ, IL-4, IL-5, IL-13, IL-1β, and TNF-α. This could be supported by the observation that treating allergic mice with SSP1-specific antibodies deceased levels many of those chemokines [26].

Present investigation also found in NPs increased expression of *C4A* and *CFB* gene encoding elements of the complement pathway. C3a and C4a are potent chemotactic factors for inflammatory cells and are able to enhance degranulation of eosinophils and mast cells which indicates its potential role in the pathogenesis of rhinosinusitis [27]. Previously Seppänen et al. [28] revealed up-regulation of complement in patients with CRS but simultaneously *C4A* deficient patients more commonly suffered from chronic or recurrent rhinosinusitis.

The role of other genes with higher transcriptional activity revealed by us in NP development is rather assumptive. The up-regulation of many of them in pathological conditions may either be the cause or a consequence of the disease. Tenascin-C is a pro-inflammatory extracellular matrix glycoprotein which appears in association with wound healing, cancer invasion, tissue remodeling and chronic inflammations [29]. Expression of tenascin-C is induced upon activation of cell surface TLRs, but also subsequent activation of TLR4 by tenascin-C stimulates the synthesis of pro-inflammatory cytokines and MMPs [30]. *PRG1* (p53-responsive gene 1) expression has been shown to be associated with accelerated cell growth, although its exact cellular function is still not clear [31]. *p22/PRG1* is a potential anti-apoptotic gene involved in NF-κB mediated resistance to apoptosis.

Attention should be paid to the function of transglutaminase 2 (TGM2). TGM2 causes a sustained increase in secretory phospholipase A(2) (sPLA(2)) activity indicating a novel mechanism by which increased expression of TGM2 may serve to amplify airway inflammation [32]. In Hallstrand et al.'s study both TGM2 gene expression and secreted protein were increased in the airways of subjects with asthma. Although TGM2 and sPLA(2)s play a major role in inflammation, their importance in chronic rhinosinusitis is not well known.

Among genes differentially expressed in

healthy nasal mucosa, the one encoding lactoferrin (LF) showed the highest (20-fold) level of transcription compared to NPs. LF represents one of the major components of mammalian secretions playing numerous roles in the innate and adaptive immunity. Moreover, LF inhibits eotaxin-stimulated eosinophil migration and through the inhibition of pro-inflammatory cytokines acts as antiproliferative factor [33]. Reports on the expression and presence of the protein in NP tissue and nasal secretion are scant and not consistent in indicating an increase or decrease in LF level in NP [34]. In view of the present outcomes and authors' previous findings the authors suppose that decreased expression of LF in NP could be one of the etiological factors disturbing the local homeostasis of nasal mucosa.

In this study the microarray technique enabled authors to identify the differential expression of NF-κB-dependent genes in NP and control mucosa. The gene expression patterns the authors obtained were significantly different in normal mucosa in comparison with NP. Consequently, all examined tissues could be classified either as NPs or healthy mucosa. Simultaneously, an analysis of NF-κB-dependent genes transcription by the described technique failed to differentiate eosinophilic from non-eosinophilic NPs, which suggests a similar expression pattern of analyzed genes in those types of NPs.

The attempt described above highlights the involvement of not only expected but, more interestingly, many unsuspected pathologic pathways. Up-regulated genes may be potentially associated with NP development. The identification of novel disease-related genes may help to understand the biology of chronic rhinosinusitis. Although NF-κB has been considered the "holy grail" in the targeting of new anti-inflammatory drugs, initial attempts did not provide satisfactory outcomes. It could be partially explained by the fact that transcription factor NF-κB influences the expression of a broad spectrum of genes, some of which may however have an opposite action during inflammation. The physiological roles of NF-κB are additionally both cell type- and stimulus-dependent. Further studies are warranted to learn more about transcriptional pathways downstream to NF-κB and the clinical usefulness of NF-κB inhibitory molecules.

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