ОГЛЯДИ ЛІТЕРАТУРИ

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УДК 616.1/4. – 002 – 008.9

IKK-IKB-NF-KB GENE MANIPULATIONS AND POLYMORPHISMS IN RELATION TO SUSCEPTIBILITY TO DIFFERENT DISEASES*

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Адаптація організмів до навколишнього середовища, механічних, хімічних та мікробіологічних впливів вимагає індуцибельної регуляції експресії генів. Одним із найвідоміших індуцибельних транскрипційних факторів є NF-kB. NF-kB має еволюційне значення не тільки для імунної системи, але й для інших органів і систем, визначаючи на експресію генів, які регулюють виживання, диференціювання і проліферацію клітин. Сигнали NF-kB, опосередковані NEMO-залежними або NEMO-незалежними IKK комплексами, слід розглядати в контексті єдиної регулюючої або сигналізуючої системи. В даному огляді ми відстежуємо деякі з важливих відкриттів, що супроводжують опис єдиної системи IKK-IKB-NF-kB для канонічної і неканонічної сигналізації. Зокрема, ми описуємо мінливість генів цієї системи та її фенотипні наслідки. Генетичні порушення цієї системи призводили до змін органогенезу і регенерації, до злоякісних пухлин, аутоімунних і запальних захворювань. Всі члени IKK-IKB-NF-kB системи можуть стати предметом як дослідження, так і маніпуляції генами. Системний генетичний підхід може стати потужним інструментом для дослідження функцій системи IKK-IKB-NF-kB і для відкриття нових діагностичних і терапевтичних стратегій.

Ключові слова: NF-kB, поліморфізми, мутація, захворювання, запалення, злоякісне новоутворення

Introduction.

The adaptation of organisms to environmental, mechanical, chemical and microbiological stresses needs inducible regulation of gene expression. One of the most well-known inducible transcription factors is NF-kB. NF-kB has evolutionarily conserved importance not only for the immune system but also for other organs and systems influencing gene expression that impact cell survival, differentiation, and proliferation. The broken NF-kB regulation leads to severe pathological conditions and diseases [1; 2; 3; 4; 5; 6]. However, despite the progress that has been made in understanding the regulation of NF-kB, there is much that remains to be understood [7]. NF-kB signalling system is involved in the development and maintenance of immune system [8], heart and vessels [9; 10], liver [11; 12], pancreas [13], muscles [14], lung [15], brain [16] etc. Number of diseases involved NF-kB system, such as: autoimmune diseases, cancer, cardiovascular disease, metabolic diseases and other [17]

There are two major pathways of NF-kB activation – inflammation with proinflammatory cytokines production [1] and malignancy with NF-kB activation accompanied by subsequent up-regulation of proliferative and anti-apoptotic agents [18].

The great interest to and importance of this transcriptional factor are displayed in *PubMed*. Searching *PubMed* for “NF-kB” yields nearly 44,100 articles (16,600 in last 5 years). This huge amount of publications concerning NF-kB has made writing of this review too difficult. Therefore, in the current review, we observe the most fundamental and partly formalized aspects of NF-kB regulation and elucidate genetic variabilities of NF-kB system that can lead to the development of numerous diseases. Of course, it is absolutely impossible to cite all primary references, used in the review but the author recommends an important set of reviews [7; 19; 20; 21; 22; 23; 24; 25; 26].

In this review we describe gene variability and its phenotypic consequences in the context of IKK-IKB-NF-kB system integrity as a useful concept for the discovery of new diagnostic and therapeutic strategies.

General information about NF-kB system. NF-kB family of transcription factors consists of effectors of a signalling system that is responsive to a large number of stimuli, mediated by most members of the tumor necrosis factor receptor (TNFR) and Toll-like receptor (TRL) superfamilies and metabolic/genotoxic stress inducers. There are three major reasons for NF-kB activation – inflammatory signals, stress response signalling and developmental (organogenic) signalling.

Inflammatory signalling. Inflammatory signals, such as cytokins (TNF-α, IL-1β), microbial products (LPS, CpG) activate NF-kB system through “classical” or “canonical” pathway. Briefly, the binding of TNFR or TLR causes phosphorylation-dependent activation of IkB kinase (IKK) complex which consists of two catalytic subunits, IKKα and IKKβ, and the scaffolding protein, IKKγ / NF-kB essential modulator (NEMO). Activated IKK complex phosphorylates IkBα,-β and-ε. IkBα is the predominant form of IkB in most cells, bound to RelA:p50 dimer. Phosphorylation of IkBβ signals the E3 ubiquitin ligase β-

* *To cite this English version: Kaidashev I. P. IKK-IKB-NF-KB gene manipulations and polymorphisms in relation to susceptibility to different diseases / / Problemy ekologii ta medytsyny. - 2014. - Vol 18, № 3-4. - P. 3 -18.*

TrCp, which catalyzes K48-linked ubiquination of IkBα leading to its subsequent degradation by the 26 S proteasome [27]. Degradation of IkBα releases RelA:p50, allowing it to localize to nucleus to bind DNA and activate gene expression.

Recent studies demonstrated that three canonical IkB proteins IkBα,-β and-ε have distinct roles in the dynamic control of NF-kB activation and termination [28]. Different inflammatory stimuli elicit different IKK activation profiles, which induce distinct temporal profiles of NF-kB activity.

Stress response signalling. Homeostatic regulation of IkB synthesis and degradation renders the NF-kB system insensitive to a variety of perturbations. The differential degradation of free and bound IkB allows for compensation between IkBs [29]. Very short half-life of free IkB necessitates a high rate of constitutive IkB synthesis to maintain a small excess of free IkB in the cell that is critical for keeping basal IkB activity levels low. Very high constitutive IkB synthesis and degradation flux provides resistance of NF-kB system to transient alterations in translation rates that are a hallmark of metabolic stress agents (ultraviolet radiation, unfolded protein response, other ribotoxic stress) [30]. DNA damage initiates the activation of the nuclear kinase ataxia telangiectasia mutated (ATM), the primary regulator of the tumor suppressor and transcription factor p53, the sumoylation of NEMO by the sumo ligase PIASy, promoting the nuclear localization of NEMO [31]. The cytoplasmic ATM-NEMO complex associates with the protein ELKS, facilitating ATM-dependent activation of the canonical IKK complex, leading to IKBα degradation and NF-kB activation [32].

Developmental (organogenic) signaling. Noninflammatory signals activate NF-kB pathway through the noncanonical NF-kB signaling. The noncanonical NF-kB signal pathway is initiated by the stimulation of TNFR superfamily with its ligands, such as B-cell activating factor (BAFF), lymphotoxin β (LTβ) and receptor activator of NF-kB ligand (RANKL). The noncanonical pathway does not involve NEMO/IKKβ-containing kinase complex but utilizes IKKα-containing kinase complex with activation of NF-kB-inducing kinase (NIK). In certain condition this pathway may crosstalk with activation of canonical one [33].

Such signals lead to the generation of the RelB:p52 dimer and RelA:p50 (via degradation of IkBδ). IkBδ activity is the result of p100 dimerization (through their RHD-interacting domains) [34]. Noncanonical signals induce the degradation of IkBδ, realizing RelA:p50 and RelB:p50 to the nucleus. Due to changes in the synthesis of p100 and p52 after 3h of stimulating the shift of predominant dimer composition from Rel:p850 and RelB:p50 to RelA:p52 and RelB:p52 occurs.

Single NF-kB system for both canonical and non-canonical signaling. Today_the numerous interconnections between canonical and non-canonical NF-kB pathways are indicated. Studies with knockout animals revealed functional overlap and interdependencies between canonical and non-canonical pathways. In the context of this review, the highly attractive position was formulated by Hoffmann et al. [20] that NF-kB signaling mediated by NEMO-dependent or NEMO-independent IKK complexes should be considered within the context of a single regulatory or signaling system [35]. Important message that the NIK, long associated with the regulation of the noncanonical pathway does in fact regulate canonical NEMO-associated IKK activity [36].

Observations suggest that NEMO-dependent and-independent signaling should be viewed within the context of a single IKK-IKB-NF-kB signaling system, which mediates signaling from both inflammatory or organogenic stimuli in an integrated manner [37; 35]. This system consists of several different IKK kinase complexes, at least 5 IKBs and 15 potential NF-kB dimeric complexes that are interdependently regulated and temporally controlled through feedback.

The recent data about the members of IKK-IKB-NF-kB signaling system are summarized in **Table 1** for clear understanding of the whole cascade.

Table 1.
IKK-IKB-NF-kB signaling system

Kinases involved in NF-KB signaling			
Gene	Polypeptide	Function	
ikbka (*Chuk*) *ikbkb* *ikbkg*	IKK complex		
	IKK1 (α) IKK2 (β) IKK γ (NEMO)	catalystic subunit catalystic subunit regulatory subunit	
nik (*Map 3k14*)	NIK	NF-KB-inducing kinase	
IkB protein family members			
Gene	Polypeptide	Stimuli that induce degradation	Receptors which bind stimuli
nfkbia *nfkbib* *nfkbie*	IkBα IkBβ IkBε	TNF, LPS, IL-1	TCR, BCR, TLR, TNF-R, IL-1R
nfkb1	p 105	LPS (in B cells)	CD14, TLR4
nfkb2	p 100	LTβ, CD40L, BAFF, RANKL, 0X40	LTβR/RANKL, CD40, BAFF-R
NF-kB family members			
Gene	Polypeptide	Possible dimers	DNA targeting activity
rela *crel* *relb* *nfkb1* *nfkb2*	RelA cRel RelB p50 p52	RelA: p50, RelA: p52, RelA: cRel, RelA: RelA, cRel: p50, cRel: p52, c Rel: cRel, RelB: p50, RelB: p52	DNA binding and activation of gene transcription
		p52: p50, p52: p52, p50: p50	DNA binding without activation of gene transcription
		RelA: cRel, RelA: RelA, cRel: cRel	Do not bind DNA

All these molecules may have different genetic variants and mutations which can influence their physiological functions. The recent data were summarized by Sun and Zhang [38].

Mis-regulation of the NF-kB pathway, either by mutation or epigenetic mechanisms, is involved in many human and animal diseases, especially ones that are associated with chronic inflammation, immunodeficiency or cancer. Mutations can be germline or somatic and include gene amplification (e.g., Rel), point mutations and deletions (Rel, NF-kB2, IKBα, NEMO) and chromosomal translocations (Bcl-3) [39].

Below we propose to discuss IKK-IKB-NF-kB signaling system in the context of two approaches: targeted

modification of genes involved and naturally occurred gene variability.

Animal models in the study of IKK-IKB-NF-KB signaling system. The generation of animal models in which individual members of gene families are genetically altered is a particularly attractive way to elucidate their function. IKB-NF-kB family and the family of kinases involved in NF-kB signaling constitute an important system of transcription factors and regulatory proteins that control the expression of cellular and viral genes crucial for a variety of processes. The findings from knockout and transgenic mice developed to study IKB-NF-kB function in vivo revealed its role in embryogenesis, development, inflammatory and stress responses [40].

ikbka

IKK1^{AA} knock-in mice spleenocytes treated with LTBR agonists had defective expression of SIC (secondary lymphoid tissue chemokine), ELC (EBI1 ligand chemokine), BLC (B lymphoblastoid cell chemokine), SDF1 (stromal cell-derived factor 1) and BAFF, so IKK1 was crucial for the chemokines and cytokines production involved in lymphoid organogenesis [41].

IKK1^{AA} mice without functional IKK1 kinase activity fail in lactation during pregnancy [42].

Knockout of IKK1 severely impaired the ability of doxorubicin to initiate NF-kappa-B-binding activity. Thus, IKK1 plays a critical role in NF-kB-mediated chemoresistance in response to doxorubicin [43].

ikbkb

To elucidate the *in vivo* function of IKK-β (IKK2), IKK2-deficient mice were generated. The homozygous mouse embryo dies due to liver degeneration and apoptosis. IKK2-deficient embryonic fibroblasts have both reduced basal NF-kB activity and impaired cytokine-induced NF-kB activation. Similarly, basal and cytokine-inducible kinase activities of the IKK complex are greatly reduced in IKK2-deficient cells. Thus, IKK2 is crucial for liver development and regulation of NF-kB activity [44]. Mouse fibroblast cells that were isolated from IKK2-/- embryos showed a clear reduction in TNF-α and interleukin-1α-induced NF-kB activity and an enhanced apoptosis in response to TNF-α. These results show that IKK2 is essential for mouse development [45].

IKK2-deficient mice have a phenotype that is remarkably similar to that of mice deficient in both the RelA (p65) and NF-kB1 (p50/p105) subunits of NF-kB. Accordingly, IKK2-deficient cells are defective in activation of IKK and NF-kB in response to either TNF-α or interleukin-1. IKK2, but not IKK1, plays the major role in IKK activation and induction of NF-kB activity. In the absence of IKK2, IKK1 is unresponsive to IKK activators [46].

Conditional gene targeting was used to evaluate the role of these proteins in B cells in adult mice. B lineage-specific disruption of IKK2-specific signals by ablation of IKK2 activity leads to the disappearance of mature B-lymphocytes. The maintenance of mature B cells depends on IKK-mediated activation of NF-kB [47]. IKK2 deficiency inhibits NF-kB activation, but does not lead to keratinocytes hyperproliferation or dysplasia. Mice with epidermis-specific deletion of IKK2 develop a severe inflammatory skin disease, which is caused by a TNF-mediated, T-cell-independent inflammatory response in the skin. The critical function of IKK2-mediated NF-kB activity in epidermal keratinocytes is to regulate mechanisms that maintain the immune homeostasis of the skin [48]. Absence of IKK2 due to knockout resulted in impaired induction of NF-kB DNA-binding activity in response to doxorubicine [43]. Deletion of IKK2 in mice with model of Duchenne muscular dystrophy led to decreasing of inflammation and muscle necrosis, and restoration of muscle fibers' regeneration through the activation of muscle progenitor cells [49].

Transgenic expression of IKK2 in mice directly increased the activity of NF-kB in pancreatic acinar cells and induced pancreatitis [50].

ikbkg

Mutant *ikbkg* (NEMO/IKKγ-deficient mice) embryos die of severe liver damage due to apoptosis. NEMO-deficient primary murine embryonic fibroblasts (MEFs) lack detectable NF-kB DNA-binding activity in response to TNF-α, IL-1, and LPS. Consistent with these data, mutant MEFs show increased sensitivity to TNF-α-induced apoptosis. Thus, NEMO/IKKγ is an essential, noncatalytic component of the IKK complex [51].

The allele of *ikbkg* in mice that impaired Toll-like receptor signaling, lymph node formation, development of memory and regulatory T cells, and immunoglobulin production was described [52].

Disruption of the X-linked gene encoding NF-kB essential modulator (NEMO) produces male embryonic lethality, completely blocks NF-kB activation by proinflammatory cytokines, and interferes with the generation and/or persistence of lymphocytes. Heterozygous female mice develop patchy skin lesions with massive granulocyte infiltration and hyperproliferation and increased apoptosis of keratinocytes. Diseased animals present severe growth retardation and early mortality. Male lethality and strikingly similar skin lesions in heterozygous females are hallmarks of the human genetic disorder incontinentia pigmenti (IP) [53].

nik

Disruption of NIK locus by gene targeting (NIK$^{-/-}$ cells) led to the abnormalities in both lymphoid tissue development and antibody responses [54].

The loss of NIK activity due to knockout (*nik*$^{-/-}$) led to functional blockade of both alternative and classical NF-kB caused by cytoplasmic retention by p100. NIK- deficient osteoclasts precursors failed to differentiate [55].

Using transgenic mice with osteoclast-lineage expressing NIK lacking its TRAF 3 binding domain, Yang et al. [56] found that NIK controls activation of alternative NF-kB pathway, a critical pathway for osteoclast differentiation. Constitutive activation of NIK drives enhanced osteoclastogenesis and bone resorption.

nik $^{-/-}$ mice were completely resistant to antigen-induced arthritis which requires intact antigen presentation and lymphocyte function. *nik* $^{-/-}$ mice were also resistant to a genetic, spontaneous form of arthritis, generated in mice expressing both the KRN T cell receptor and H-2. Thus, *nik* is important in the immune and bone-destructive components of inflammatory arthritis and represents a possible therapeutic target for these diseases [57].

nic$^{-/-}$ mice had a complex phenotype consisting of immunosuppressions mediated by CD25^{-} Foxp3^{-} memory CD4^{+} cells and, in the absence of those cells, hyperresponsive naïve CD4^{+} T cells which caused autoimmune lesions after adoptive transfer into hosts deficient in recombination-activating genes [58]. These findings showed the importance of p100 for the activation of naïve T cells.

A point mutation causing an amino acid substitution in the carboxy-terminal interaction domain of NIK results in autosomal recessive alymphoplasia (aly) in mice. This

disorder is characterized by the systemic absence of lymph nodes and Peyer's patches, disorganized splenic and thymic structures with immunodeficiency [59]. Also aly/aly mice had the reduced serum levels of immunoglobulins and the absence of class switch to IgA [60].

A natural mutation of the gene encoding NIK in aly mice cripples the function of NIK in p100 processing, causing a severe defect in p52 production [61].

nfkbia

nfkbia knockouts result in lethality 7-10 days after birth due to hyperinflammation [62]. Mutation in kB enhancers of *nfkbia* promoter led to short lifespan, hypersensitivity to septic shock and abnormal T-cells development and attraction [63].

For investigation of IKBα the transgenic mice expressing the IKB-α S32/36A superrepressor protein under control of the mouse mammary tumor virus long terminal repeat promoter were generated. It was shown that mice had a transient delay in mammary ductal branching. Thus, IKBα and consequent activation of RelB/p52 involved in mammary gland development and carcinogenesis [64].

Mice with pancreas-specific deletion of *nfkbia* had constitutive activation of RelA and a gene expression profile consistent with NF-kB activation; development of acute pancreatitis in these mice was attenuated and trypsin activation was impaired [65].

nfkbib

Absence of IkBβ results in a dramatic reduction of TNF-α in response to LPS even though activation of NF-kB is normal. As a result, *nfkbib*$^{-/-}$ mice are resistant to LPS-induced septic shock and collagen-induced arthritis [66].

IκBβ-deficient mice proved to be highly refractory to LPS-induced lethality, accompanied by a strong reduction in sepsis-associated cytokine production. Further transcriptome analysis of LPS-stimulated wild-type and IkBβ-deficient bone marrow-derived macrophages revealed several other genes with known regulatory functions in innate immunity arguing that a subset of NF-kB target genes (including IL-1) is under control of IkBβ [67].

In the absence of IkBβ, IkB-α or other inhibitory proteins can regulate NF-kB functions essential to acute neutrophil emigration in the lungs [68].

nfkbie

nfkbie knockout mice are not lethal, but result in the increased expression of IL-1 alpha, IL-1β, IL-1Ra and IL-6 mRNA in contrast to GM-CSF, C-CSF, and IFN-γ which remain undetectable. Also 50% reduction of the $CD44^{-}$ $CD25^{+}$ T cell subspecies was shown in mutant mice. Knockout mice presented constitutive up-regulation of IgM and IgG1 isotypes [69].

Neither IKBα nor IKBε deficiency had major effects on NK cell generation, while their combined absence led to NF-kB hyperactivation, resulting in reduced NK cell numbers, incomplete NK cell maturation, and defective IFN-γ production [70].

nfkb1

Mice lacking the p50 subunit of NF-kB1 showed no developmental abnormalities, but exhibited multifocal defects in immune responses involving B lymphocytes and non-specific responses to infections. These mice were unable to clear L. *monocytogenes* and were more susceptible to S. *pneumonia* [71].

Deletion of p50 led to the extent of expansive remodelling and aggravated systolic dysfunction increasing of interstitial fibrosis and hypertrophy in the noninfarcted myocardium and inflammation in mice with myocardial ischemia [72].

In contrast, gene deletion of p50 in mice does not alter the hepatic inflammatory response to ischemia/reperfusion. Despite abrogation of DNA-binding by NF-kB p50/p65 complex, p65 was still observed in nuclear extracts suggesting that there may be functional redundancy amongst members of the Rel protein family in order to preserve the inflammatory response [73]. $p50^{-/-}$ mice demonstrated the enhanced premature cytoxicity of murine embryonic fibroblasts infected by murine encephalomyocarditis virus. These results showed that p50 is equally important in suppressing apoptosis during viral infection [74].

NF-kB1 deficiency in OTII cells (mouse OVA-specific $CD4^{+}$ T cells) results in impairment of IL-4 and IL-13 production and expression of CXCR5. These results suggest that NF-kB1 regulates the expression of CXCR5 on $CD4^{+}$ T cells primed in vivo, and thus selectively controls the B-cell response to alum OVA [75].

Yang et al. [76] found that in *nfkb1* (SSAA/SSAA) mice in which IKK target serines on p105 are mutated to alanines the agonist-induced release of TPL-2 kinase from its inhibitor p105 was prevented. The *nfkb1* (SSAA) mutation also prevented LPS-induced processing of p105 to p50 and reduced p50 levels. This *nfkb1* mutation resulted in less activation of NF-kB in $CD4^{+}$ T cells and proliferation of $CD4^{+}$ T cells after stimulation of the T cell antigen receptor. So, IKK-induced p105 proteolysis was therefore essential for optimal T cell antigen receptor-induced activation of NF-kB and mature $CD4^{+}$ T cell function [77].

nfkb2

nfkb2$^{-/-}$ mice showed a marked reduction in the B cell compartment in spleen, bone marrow, and lymph nodes. Spleen and lymph nodes of mutant mice presented on altered architecture with diffuse, irregular B cell areas and the absence of discrete perifollicular marginal and mantle zones. These animals presented a deficient immune response to T cell-dependent and-independent antigens [78]. p52 null mutant mice were impaired in their ability to generate antibodies to T-dependent antigens, consistent with an absence of B cell follicles and follicular dendritic cell networks in secondary lymphoid organs, and the inability to form germinal centers [79].

In *nfkb2*$^{-/-}$ mice microscopic inspection showed the absence of detectable Peyer's patches. Whole-mount in situ hybridization revealed the presence of IL-7 receptor-alpha spots in these mice, indicating no defect in Peyer's patches organogenesis in principle. Immunostaining showed that residual lymphocytes mainly consisted of T cells. B cells were reduced and accumulated as terminal extravasations. Organized follicular structures and follicular dendritic cell networks fail to form, and myeloid, but not lymphoid dendritic cells were obviously reduced. Expression of several chemokines was impaired in epithelial cells and in the subendothelial dome area that was not well defined [80]. In NF-kB2, encoding p100/p52, deficient mice the development of Peyer's patches was impaired [81].

Tucker et al. [82] generated novel mutation in *nfkb2* that prevents the processing of the inhibitory precursor, p100, into the active subunit, p52. Mutant mice express a complex phenotype with abnormalities in a variety of tissues and with a spectrum that is more severe than in mice carrying a targeted deletion of *nfkb2*. Thus, NF-kB2 had a key role in the regulation of RelA activation (with p100 and p52 production).

T cells from *nfkb2*$^{-/-}$ mice which cannot generate the p52 component of noncanonical NF-kB2 were also costimulation independent, consistent with the negative role of this unprocessed protein in canonical NF-kB activation [83].

In transgenic mouse model with lymphocyte-targeted expression of p80HT, a lymphoma-associated NF-kB2 mutant, approximately 40% of mice showed elevated levels of monoclonal immunoglobulin in the serum and developed plasma cell tumors. B cells from these mice revealed affected survival and aberrant expression of cyclin D1, cyclin D2, IL-10 and IL-15 [84].

The generation of transgenic mice with targeted expression of p80HT, a lymphoma-associated NF-KB2 mutant, in lymphocytes led to marked expansion of peripheral B cell populations and develops predominantly small B cell lymphomas. These B cells showed specific resistance to apoptosis induced by cytokines deprivation and mitogenic stimulation [85].

In contrast to *nfkb2*$^{-/-}$ mice, which lack both p100 and p52, mice that lack only the inhibitory p100 precursor but still express the p52 subunit of NF-KB2 (p100$^{-/-}$) had markedly elevated the splenic marginal zone B cell numbers. Both cell-intrinsic mechanisms and increased stromal expression of vascular cell adhesion molecule-1 (VCAM-1) contributed to the accumulation of B cells in the marginal zone in p100$^{-/-}$ spleens. p100 deficiency resulted in the absence of normal marginal sinus, strongly induced expression of mucosal addressing cell adhesion molecule-1 (MAdCAM)-1 and glycosylated cell adhesion molecule-1 (GlyCAM-1), and the formation of nonfunctional ectopic high endothelial venule (HEV)-like structures in the red pulps [86].

Mice lacking p100 (p100$^{-/-}$) but still containing a functional p52 protein had marked gastric hyperplasia, resulting in early postnatal death, alterations of hemotopoietic tissues, enlarged lymph nodes, increased lymphocyte proliferation, enhanced cytokine production in activated T cells. These data supposed that p100 was essential for the proper regulation of p52-containing Rel/NF-kB complexes [87].

In C57BL/6 mice a mutant strain with selective deficiency in recirculating B cells but not immature or peritoneal B1 cells was associated with a point mutation in the gene encoding NF-KB2, terminating the encoded protein within the DNA-binding domain. The mutation absence of p100 affects a cell autonomous process within B cells that is required for their accumulation after emigrating to peripheral lymphoid organs [88].

rela

In *rela*$^{-/-}$ mice deficiency of RelA/p65 protein results in embryonic lethality due to massive apoptosis in the fetal liver [89].

The *rel*$^{-/-}$ *tnfr*1$^{-/-}$ genotype results in defects in the immune system, confirming the requirement of RelA for the functioning of immune and inflammatory responses in cells [90].

crel$^{-/-}$ *rela*$^{-/-}$ mutation led to more severe disorders of innate immune-mediated inflammation than either of the single gene knockouts [91].

Immortalized mouse embryonic fibroblast cell lines prepared from RelA knockout mice had different phenotypes, based on their sensitivity to TNF-α-induced apoptosis, morphology, ability to form colonies in soft agar, and the presence of distinct kB site-binding complexes. These cell lines appear to have distinct alterations in the p53 pathway [92]. RelA-deficient mice showed the absence of Peyer's patches, lymph nodes and disorders of splenic microarchitecture and had a profound defect in T cell–dependent antigen responses [93].

Transgenic expression of p65 led to compensatory expression of the inhibitory subunit IKBα and, therefore, no clear phenotype. However, p65 transgenic mice given injections of cerulean, to induce acute pancreatitis, had higher levels of NF-kB activity in acinar cells, greater levels of inflammation, and more severe outcomes than control mice [50].

Knockdown of p65 in MDA-MB-468 breast cancer cells expressing recombinant transglutaminase 2 partially reduces resistance to doxorubicin, indicating that the drug resistance linked to overexpression of transglutaminase, through p65 [94].

Mutation of 41R and 42S in the Rel homology domain of p65 facilitate the interaction with the basal transcription factor IIB (TFIIB) [95].

The elimination in mice of both p65 (RelA) and STAT3, but neither alone, abrogated all acute phase responses measured. The failure to respond was consistent across multiple different infectious, inflammatory and noxious stimuli, including pneumococcal pneumonia [96].

Mutation of the threonine 505 (T505) phosphosite to alanine in p65 has wide-ranging effects on NF-kB function in mice. These include effects on chemotherapeutic drug-induced apoptosis and roles for this modification in autophagy, cell proliferation and migration [97]. Loss p65 decreased average spine head volume; all classes of dendritic spines in pyramidal neurons had smaller head diameters. These effects were consistent with weakened excitatory synaptic connectivity and fewer mature dendritic spines in the absence of p65 [98]. Ablation of 1 allele of the p65 was sufficient to improve pathology in mouse model of Duchenne muscular dystrophy with elevated IKK/NF-kB signaling [49].

Transfection with p65 siRNA attenuated the elastase-induced nuclear translocation of p65 NF-kB/Rel A, upregulation of Fas/FasL, caspase-3, DNA fragmentation, and apoptosis of Kupfer cells in NIH Swiss mice with acute pancreatitis [99].

crel

In domain mutation studies the removal of C-terminal activation domain of cRel led to enlarged lymph nodes and lymphoid hyperplasia [100].

crel$^{-/-}$ mice are not lethal and show several defects in the cell cycle progression and survival in B cells, defects in CD4 and CD8 T-cell responses, impaired cytokine production [101]. Generation of doubly deficient p50$^{-/-}$ *crel*$^{-/-}$ mice revealed that dendritic cells (DC3) developed normally, but CD40L^{-} and TRANCE-induced survival and IL-12 production was abolished [102].

crel$^{-/-}$ mice display a defect in the neutrophilic inflammatory response, associated with impaired induction of RANTES. The fibrogenic / wound-healing response to injury was also impaired and this was associated with deficiencies in the expression of fibrogenic genes, collagen I and alpha-smooth muscle actin. cRel deficient mice have smaller hearts at birth as well as during adulthood, and are protected from developing cardiac hypertrophy and fibrosis after chronic angiotensin infusion [103].

Absence of cRel was associated with blunted and delayed induction of forkhead box M1 (FoxM1) and its downstream targets cyclin B1 and Cdc 25C [104].

cRel partially compensates for the loss of RelA in *rela*$^{-/-}$ mice, suggesting that cRel and RelA have overlapping functions at least in fetal liver cells [105].

relb

relb $^{-/-}$ mice are unstable to form germinal centers and follicular dendritic cell networks upon antigen chal-

lenge in the spleen. Expression of homing chemokines is strongly reduced in *relb*$^{-/-}$ spleen with particularly low mRNA levels of the chemokine B lymphocyte chemoattractant [106]. Mice had phenotypic abnormalities including multifocal, mixed inflammatory cell infiltration in several organs, myeloid hyperplasia, spleenomegaly due to extramedullary hematopoiesis, and a reduced population of thymic dendritic cells [107]. In RelB null mice was shown that RelB selectively regulates a myeloid-related dendritic cell lineage [108].

It was demonstrated that *relb* $^{-/-}$ cells have decreased amounts of p100 protein and *nfkb2* $^{-/-}$ showed reduced level of RelB protein. Targeted disruption of RelB in mice results in anatomical defects of secondary lymphoid tissues. Development of Peyer's patch-organizing centers is impaired in RelB-deficient mice [81].

Synthesis control of RelB is the major determinant of noncanonical NF-kB dimer activation. Processing, not synthesis, of p100 and p105 is mechanistically linked via competitive dimerization with a limited pool of RelA and RelB. RelB was needed for the gene expression of SLC, ELC, BLC, SDF-1, BAFF in *relb* $^{-/-}$ and *nfkb2* $^{-/-}$ cells [109].

relb$^{-/-}$ fibroblasts have profound alterations of circadian genes expression. These findings revealed function for RelB as an important regulation of the mammalian circadian system in fibroblasts [110].

RelB silencing by small interfering RNA in dendritic cells led to maintain their immature status and slightly impaired immune surveillance of T cells [111].

Clinical observations of IKK-IkB-NF-kB genetic variability

ikbka

Recent data showed some gene variation in human *Ikbka* [112]. In literature there are few studies of association ikbka polymorphisms and clinical disorders. For example, there was no any major effect of *ikbka* polymorphism on the NF-kB pathway in rheumatoid arthritis (RA) susceptibility [113].

ikbkb

Recent evidence indicates that IKK2 may be a mediator of acquired forms of insulin-resistance. Linkage with four markers flanking the *ikbkb* gene was evaluated in 32 multigenerational families. Polymorphisms were identified in the 5' flanking region of *ikbkb* (-1775del/insC and-1547T > A), exon 11 (c.1083A > G, L361L) and in intron 12 (IVS12+14t > a). Results reflected that sequence differences in the *ikbkb* gene do not play a major role in either early-onset, autosomal dominant type 2 diabetes, or common forms with a later-onset [114].

The multilocus associations of inflammation genes and colorectal cancer risk were studied by the hapConstructor method to a multilocus investigation of candidate genes. The most significant finding was a combined genotype association across *ikbkb* SNP rs5029748 (1 or 2 variant alleles), *IL6* rs1800797 (1 or 2 variant alleles), and *nfkb1* rs4648110 (2 variant alleles) which conferred an ~80% decreased risk of colon cancer [115].

ikbkg

The range of diseases caused by NEMO mutations highlights the physiological importance of NEMO and the IKK complex. Null alleles of the x-linked gene encoding NEMO, IKBKG, cause the inflammatory skin disease incontinentia pigmenti in heterozygous females, and are lethal in heterozygous males, as they are in mice [116; 117; 51; 53].

Milder hypomorphic alleles are compatible with viability in males, but cause severe immune deficiency and developmental abnormalities of the teeth, hair, or sweat glands [118]. These abnormalities of ectodermal derivates are thought to result from disruption of EDAR signaling, yet there are reports of *ikbkg* mutations in immune-deficient patients without ectodermal dysplasia [119; 120]. Other mutations appear to disrupt EDAR signaling and CD40-mediated immunoglobulin-class switching but not TLR signaling, whereas another mutation disrupts EDAR signaling, another mutation disrupt EDAR signaling, but leaves TLR and CD40 signaling largely intact [121; 122].

Mutation in the 5' untranslated region of the *ikbkg* gene led to the immune deficiency in patient (recurrent sinopulmonary infections and dysgammaglobilinemia) due to defect in the p65 nuclear translocation [123].

N-terminal deletion of IKKγ (to inhibit the IKK complex) delayed growth kinetics, caused morphological changes and dramatically augmented apoptosis in keratinocytes [124]. Hypomorphic mutations in the zink finger domain of NEMO cause x-linked hyper-IgM syndrome with ectodermal dysplasia. The patient B cells stimulated with CD40 ligand are impaired in both p65 and cRel activation and whereas adaptation of IL-4 can enhance p65 activity, cRel activity remains deficient [125].

Ørstavik et al. [126] described the novel splicing mutation in *ikbkg* gene with severe immunodeficiency and heterogeneity of x-chromosome inactivation.

nik (Map3k14)

SNP array analysis revealed a gain of copy number for MAP3K14 in three classical Hodgkin lymphoma cell lines [127].

The CC genotype of *nik* (rs7222094) is associated with increased mortality and organ dysfunction in septic shock patients, perhaps due to altered regulation of NF-kB pathway genes, including CXCL10 [128].

The evidence of association with bone mineral density, bone geometric parameters and CTX-I (bone turnover marker) was found for SNPs in *nik* in 2359 men aged 40-79 years [129]. rs4792847 (*Map3k14*) showed evidence of association to treatment response in a large cohort of RA patients and were subsequently examined in an independent cohort of patients [130].

nfkbia

X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by hypomorphic mutations in *ikbkg* encoding NEMO/IKK gamma, the regulatory subunit of the IKB kinase (IKK) complex. Also an autosomal-dominant form of ectodermal dysplasia with immunodeficiency with heterozygous missense mutation at serine 32 of IKBα was described. This mutation is gain-of-function as it enhances the inhibitory capacity of IKBα by preventing its phosphorylation and degradation and results in impaired NF-kB activation. This disorder is characterized by a severe and unique T cell immunodeficiency-marked blood lymphocytosis, absence of detectable memory T cells, naïve T cells do not respond to CD3-TCR activity in vitro [131; 132].

Novel heterozygous mutation at amino acid 11 (W11X) IKB α was described in female patient with ectodermal dysplasia with immune deficiency. This mutant protein did not undergo ligand-induced phosphorylation or degradation and retained NF-kB in the cytoplasm [133]. Fibroblasts isolated from a patient with growth failure and a heterozygous mutation of IKBα exhibit growth hormone insensitivity [134].

Some IKBα mutants – IKBα M (amino acids 1-317, Ser32, 36A), IKBα 243 N (amino acids 1-243), IKBα 244 C (amino acids 244-317) were constructed and transfected to ASTC-α-1 cells. IKBα M bounds with NF-kB and p53 in cytoplasm steadily, and inhibits both of the two signaling pathways. IKBα 244C may be co-factor in inducing apoptosis. The C-terminal of IKBα enhanced cell death [135].

In Hodgkin/Reed-Sternberg cells from Hodgkin's disease patients the mutations in the IKBα were detected. These mutations resulting in C-terminally truncated proteins were not able to inhibit NF-kB-DNA binding activity [136]. Inactivating mutation of *nfkbia* was described in patient with classic Hodgkin lymphoma. Multiple lesions in regulations of NF-kB signal pathway can likely cooperatively contribute to the strong NF-kB activity of lymphoma cells [137].

Hatta et al. [138] described a mutational analysis of IKBα for primary tumor cells obtained from patients with a variety of hematologic malignancies (acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, hairy cell leukemia, adult T-cell leukemia and mantle cell leukemia) as well as 15 leukemia, lymphoma, and myeloma cell lines. Authors supposed that mutations of IKBα could be rare events in these diseases, except for Hodgkin's lymphoma.

Mutations that cause premature termination of translation in three of the four copies on *nfkbia* revealed that absence of IKBα protein in human B-cell lymphoma cell line RC-K8. Also RC-K8 cells have a rearranged Rel locus that directs the production of chimeric protein, termed Rel-NRG (Non-Rel gene). In vivo, Rel-NRG cannot activate transcription of kB site reporter plasmid, suggesting that is a transcription regressing or blocking Rel protein [139].

nfkbia is often deleted but not mutated in glioblastomas, most deletions occur in nonclassical subtypes of the disease. Deletion of *nfkbia* and amplification of epidermal growth factor receptor (EGFR) show a pattern of mutual exclusivity. Restoration of the expression of nfkbia attenuated the malignant phenotype and increased the vulnerability to chemotherapy of cells. Thus, deletion of nfkbia has an effect that is similar to the effect of EGFR amplification in the pathogenesis of glioblastoma [140].

A full *nfkbia* gene was sequenced and nine novel SNPs and one GAA deletion were identified. There was no significant association of *nfkbia* variants with development of hepatocellular carcinoma among chronic hepatitis B patients [141]. These data were supported by Cheng et al. [142].

Recipients SNPs of *nfkbia* gene together with other genes SNPs can be used for clinical outcomes prognosis after allogenic stem-cell transplantation [143].

Three Canadian family-based studies and 1 Australian population-based case-control study were used to investigate association of 321 SNPs in 26 innate immunity genes with atopy, asthma, atopic asthma and airway hyperresponsiveness. *nfkbia* SNP was associated with atopic asthma [144].

The association between *nfkbia* polymorphisms and acute lung injury was observed in patients with severe trauma [145].

nfkbia polymorphism (-826T (CT+TT) and-881G (AG+GG)) seems to be related to susceptibility to develop oral cancer linked to betel nut and tobacco consumption. Patients with oral cancer who had at least one-519 T allele of *nfkbia* gene were at higher risk for developing distant metastasis, compared with those patients CC homozygotes [146].

The association between coding (non synonymous and synonymous) polymorphisms of *nfkbia* and other genes with measles-specific IL-2, IL-6, IFN-α, IFN-γ, IFN-λ-1 and TNF-α during measles vaccine immunity was described [147]. It was shown that *nfkbia* polymorphisms associate with susceptibility to invasive pneumococcal disease. Rare *nfkbia* mutations cause immunodeficiency with severe bacterial infection [148].

Meta-analysis of 14 studies in *PudMed* revealed that autoimmune and inflammatory diseases are associated with *nfkbia* gene – 826 C/T polymorphism, but not with 2758 A/G,- 881 A/G/, and – 279 C/T [149]. Association between *nfkbia* SNPs and Graves' disease was not found in the investigation of 481 patients and 455 healthy controls [150]. *nfkbia* 3' UTR AA genotype associated with Crohn's diseases and GG genotype with an increased risk for extensive colitis in Hungarian patients [151].

Chinese individuals ≥ 50 years of age carrying AG genotype (rs 696) of *nfkbia* may be at a risk of developing colorectal cancer and the GG genotype may be considered as a prognostic factor for Swedish Colorectal cancer patients [152]. The association of the AA genotype of *nfkbia* gene has been found latent autoimmune diabetes in adults [153]. The haplotype GTC (-881G/- 826t/- 297 c) of *nfkbia* is associated with higher risk of acute respiratory distress syndrome in Caucasians, particularly in male patients and in patients with direct lung injury [154]. The evidences were provided for associations of SNPs in *nfkbia* with severe carotid artery disease [155]. The AA genotype of *nfkbia* gene presents a risk for type 2 diabetes mellitus development but not for diabetic nephropathy alone [156]. A risk haplotype [GCCTATCA] of eight polymorphisms across *nfkbia* gene for multiple myeloma was identified [157].

nfkbib

The novel polymorphism of *nfkbib* (rs 3136641) associated with early viral infections and susceptibility to asthma and asthma related phenotypes [144]. COPD phenotype significantly associated with *nfkbib*/*sirt2* polymorphism (rs 2241704) [158]. Polymorphism of *nfkbib* did not reveal connection to ovarian cancer risk [159].

nfkbie

An *nfkbie* SNP associated with susceptibility to invasive pneumococcal disease [148]. Two *nfkbie* genes loci associated with rheumatoid arthritis susceptibility at 6p21.1, rs 2233434, and rs 77986492 were recognized in Japanese [160].

Similar data about relation of *nfkbie* genetic variation with susceptibility to RA were obtained in Spanish patients [113]. In patient with chronic lymphocytic leukemia the mutation of *nfkbie* was identified in cases with progressive disease [161].

nfkb1

NF-kB was mapped with greater resolution to 4q23 and these regions associated with certain types of acute lymphoblastic leukemia [162].

Karban et al. [163] indentified a common insertion/deletion promoter polymorphism (-94 ins/del ATTG, rs 28362491) in *nfkb1*(encodes p50 subunit), and provided evidence for functionality in a reporter assay as well as an association with ulcerative colitis. The same polymorphism is associated with superficial bladder cancer and melanoma [164; 165].

Association of this *nfkb1* gene polymorphism (the presence of deletion allele) with risk of alcohol liver cirrhosis was found in patients with alcohol dependence [166]. This polymorphism modifies the association between dietary polyunsaturated fatty acid intake and circulating HDL-cholesterol [167]. The meta-analysis (2,743 cases and 2,195 controls) demonstrated the association of *nfkb1*-94 ins/del ATTG polymorphism with cancer in Caucasian and Asian populations, and this association is ethno-specific [168]. A genetic association study revealed

the-94 ins/del ATTG polymorphism was positively associated both with moderate/severe endometriosis and idiopathic infertility [169]. Patients with RA carrying the del/del genotype had higher risk of cardiovascular events than those with ins/ins genotype, while heterozygous patients had the intermediate risk [170]. A meta-analysis of seventeen studies did not found the association between this polymorphism and autoimmune and inflammatory diseases in the Caucasian population. However, an association was found in the Asian population [171].-94 ins/del ATTG *nfkb1* gene variant may contribute to lower myocardial infarction susceptibility via the potential reduction of activated NF-kB which in turn is related to plasma inflammatory marker (fibrinogen, C-reactive protein) reduction [172]. This polymorphism results in lower protein levels of NF-kB p 50 subunits. Nfkb1 ATTG(1) / ATTG(1) genotype was significantly associated with left ventricular dysfunction [173]. Also, this polymorphism contributes to the susceptibility of congenital heart diseases [174], increased mortality in sepsis [175], increased risk of renal cell carcinoma [176].

The *nfkb1* polymorphism-449 C > G (rs 72696119) in 5'-UTR was significantly associated with the development of ulcerative colitis [177].

Risk of non-Hodgkin's lymphoma is associated with *nfkb1* intronic tag SNP (rs 4648022) [178].

15 alleles for the *nfkb1* gene polymorphism (CA-repeats) were detected in Czech population. The alleles were ranging in size from 114-142 bp corresponding 10-25 CA repeats. Frequency of the A7 allele of *nfkb1* gene has been significantly increased in adults with diabetes mellitus type 1 [153].

Also, a significant combined effect of rs 3774959 and rs 3774964 in the *nfkb1* gene with rs 222991 in the REST (RE-A-silencing transcription factor) gene was associated with the risk of colorectal cancer [179]. Jiaox et al. [180] observed somatic mutations of NFKB1 in the preparations of 96 human breast cancers. In multiple myeloma mutations of nfkb1 selectively activate the classical NF-kB pathway [181].

In sporadic Parkinson's diseases patients detailed mutation analysis of the p50 subunit of nfkb1 did not reveal definable role of *nfkb1* polymorphism in the pathogenesis of these diseases [182].

nfkb2

In general, mutant NF-kB2 proteins can lead to the transformed phenotype and alterations in *nfkb2* may play role in lymphomagenesis [183].

Several chromosomal aberrations affecting *nfkb2* were described. In multiple myeloma, cloning and sequencing analysis of reciprocal breakpoint sites showed that they occurred within intron 15 of *nfkb2* and led to the complete deletion of 3' portion of the gene coding for the ankyrin domain. The novel regions involved in nfkb2 rearrangement originated from chromosome 7q 34, thus implying the occurrence of a t (7;10) (q34; q24) reciprocal chromosomal translocation. In T cells cutaneous lymphoma and B cell chronic lymphocytic leukemia, *nfkb2* rearrangements occurred, respectively, within exon 18 and 20 of the gene and involved recombinations with distinct regions of chromosome 10q 24. Rearrangement led to specific C-terminal truncations of NF-kB2 generating abnormal transcripts that coded for proteins lacking of the ankyrin domain that may be involved in tumorgenesis [184].

The new point mutation of p100 that is encoded by *nfkb2*, called p100 HB generates a premature stop-codon and thus the protein lacks the last 125 amino acids. This mutation was detected in several human tumor cell lines. Mutant protein has reduced inhibitory potency compared to p100 and translocates into the nucleus [185]. Constitutive processing of p100 occurs in certain lymphoma cells due to the loss of its C-terminal regulatory domain. The constitutive processing of C-terminal truncation mutants of p100 is associated with their active nuclear translocations. Mutation of the nuclear localization signal (NLS) of p100 abolishes its processing, and this defect can be rescued by fusion of a heterologous NLS to the animo- or carboxyl-terminus of the p100 mutant [186].

A C terminally-truncated form of NF-KB2 p100 (p85), produced in HUT-78 human leukemic cells, also activated transcription in yeast, under conditions where the normal p52 and p100 were not [187].

A rearranged *nfkb2*/p100 gene was isolated from adult T-cell leukemia-derived cell line, which was generated by a chromosomal translocation [188]. Acute lymphoblastic leukemia (ALL) might be associated with novel translocations found in the leukemic cells of ALL patients – t (5;10) (q 22; q 24). FISH and Southern blot hybridization studies have eliminated likely involvement of *nfkb2* on chromosome 10 [189]. It was shown that *nfkb2* gene duplication is associated with fetal pyelectasis in partial trisomy 10g (10 g 24.1→qter) [190].

In Japanese population three polymorphisms of *nfkb2* gene (1837T/C, 1867G/G in upstream region and 2584G/T within intron 1) is associated with inflammatory response and bone differentiation [191].

rela

Structural alterations of *rela* gene may represent rare events in lymphoid neoplasia. By means of PCR-SSCP analysis a single point mutation leading to amino acid substitution (codon 494, Glu-Asp) in the transactivating domain in one case of multiple myeloma was detected. This mutation may alter the specific structural conformation needed for the DNA interaction of RelA [192].

A novel member of the human NF-kB family, denoted RelA p43, the nucleotide sequence of which contains several exons as well as an intron of the RelA gene, was identified. p43 is expressed in all cell lines and tissues, exhibiting all the properties of a NF-kB proteins. Its sequence does not include a transactivation domain but it is able to potentiate RelA-mediated transactivation and stabilize dimmers comprising p50 [193].

Cai et al. [194] did not find any associations of 5 *rela* SPNs with pulmonary tuberculosis in the Chinese Han population.

Mutation of p65 at Ser 468 largely prevents p65 ubiquitinylation and proteasomal degradation. Phosphorylation of p65 at Ser 468 leads to ubiquitin proteasome-dependent removal of chromating-bound p 65, thus contributing to the selective termination of NF-kB-dependent gene expression [195]. The mutation S276A of p65 affected the expression of several genes that encode proteins involved in cell cycle regulation, signal transduction, transcription and metabolism [196]. Mutation Cys 38 Ser of p65 abolished the suppressive effect of picroliv on NF-kB-regulated gene products, and apoptosis enhancing. Thus, Cys 38 was important for phosphorylation and nuclear translocation of p65 [197]. Mutation S276A of p65 led to the inhibition of Schwann cell differentiation into a myelinating phenotype [198].

Three SNPs in the *rela* gene (rs11850062, rs 2306365, and rs 7119750) are significantly associated with schizophrenia [199].

crel

Starczynowski et al. [200] described point mutation of *crel* that changes serin (Ser) 525 (TCA) to proline (Pro) (CCA) within the cRel transactivation domain. This mutation was of germ-line origin and was identified in two human B-cell lymphomas. cRel-S525P had a reduced ability to activate the human manganese superoxide dismutase promoter and a reduced IKKα- and TNF-α-stimulated transactivation by GAL4-Rel protein.

A genome-wide association study of Hodgkin's lymphoma revealed new susceptibility locus at 2p 16.1 (rs 1432295, REL) [201]. A genome-wide association study of rheumatoid arthritis (RA) in 2418 cases and 4504 controls from North America identified an association at the REL locus, encoding *crel*, on chromosome 2p13 rs 13031237 and rs 2736340 [202]. Later significant evidence for association with susceptibility to RA was found to a SNP mapping tp the REL (rs 13017599) gene [203].

Psoriatic arthritis is associated with 2p16 near the REL locus. rs 13017599 and rs 702873 had significant link to psoriatic arthritis [204]. A genome-wide association between celiac disease and risk region 2p 16.1 (REL) (rs842647) was established [205]. The association of SNP REL (rs 842647) with celiac disease was identified in 157 Italian families [206]. For ulcerative colitis SNPs at chromosome 9q34 were determined to suppose REL as the putative candidate gene [207]. The Rel locus rs1303123 is strongly associated with rheumatoid arthritis in US, UK and Canadian populations [208].

relb

We have found the lack of publications about the relb polymorphisms/mutations in human.

Detectable nasopharyngeal shedding of severe acute respiratory syndrome (SARS)-associated coronavirus was associated with a member of NF-kB complex (reticuloendotheliosis viral oncogene homolog B [RelB]) [209].

General consideration on IKK-IKB-NF-kB Gene Variability Consequences. In table 2 we briefly summarized all data from animal models and clinical observations of IKK-IKB-NF-kB system gene variability.

Table 2.
IKK-IKB-NF-kB Gene Variability Consequences

Protein family	Gene	Normal phenotype and disorders
IKK complex	*ikbka*	Important for lymphoid and gland organogenesis (impair production of chemo- and cytokines involved in lymphoid organogenesis, lactation disorders during pregnancy)
	ikbkb	Crucial for liver development and regulation of NF-kB activity, development of mature B cells, regulation of immune homeostasis in the skin (liver degeneration and apoptosis, death as embryos, disappearance of B lymphocytes, severe epidermal inflammation due to TNF-mediated response)
	ikbkg	Liver development (lack of detectable NF-kB DNA-binding activity, severe liver damage due to apoptosis)
NIK	*nik*	Lymphoid tissue development and antibody responses (defect in secondary lymphoid tissue chemokine receptor signaling and homing) Alymphoplasia in mice (absence of lymph nodes and Peyer's patches, disorganized splenic and thymic structures with immunodeficiency, severe defect in p52 production) Osteoclastogenesis (blockade of both alternative and classic NF-kB activation, impaired osteoclast differentiation)
IKB protein family members	*nfkbia*	Embryo- and ontogenesis (lethality 7-10 days after birth), hyperinflammation, hypersensitivity to septic shock, delay in mammary ductal branching Abnormality in T cells development and attraction Mammary gland development and carcinogenesis Activation of NF-kB in pancreas lead to acute pancreatitis
	nfkbib	Cytokine production (strong reduction in sepsis-associated cytokine production after LPS stimulation, high refractory to LPS-induced lethality)
	nfkbie	Cytokine and antibody production (increased expression of IL-1α and IL-1β, IL-1Rα and IL-6 mRNA; up-regulation of IgM and IgG1 isotypes)
NF-kB family members	*nfkb1*	Cytokine production, B cell regulation (impairment of IL-4 and IL-13 production and CXCR5 expression on $CD4^+$ cells)
	nfkb2	Lymphoid organs development, antibody production, cytokines expression (Peyer's patches abnormalities, reduction of B cell compartments in spleen, bone marrow and lymph nodes, impair production of antibodies to T-depended antigens, absence of DC network, lymphoma, multiple myeloma, T cell cutaneous lymphoma, B cell chronic lymphocytic leukemia, inflammatory response, bone differentiation)
	rela	Lymphoid neoplasia, multiple myeloma, impaired expression of genes that encode proteins involved in cell cycle regulation, signal transduction, transcription and metabolism. Myelination inhibition, schizophrenia.
	crel	B-cell lymphomas, rheumatoid arthritis, psoriatic arthritis, celiac disease, ulcerative colitis
	relb	Severe acute respiratory syndrome (SARS)

Kinases involved in NF-kB signaling are highly important for tissue development, proliferation, apoptosis and inflammation. *ikbka* had crucial role in lymphoid and gland organogenesis. Liver development and regulation of NF-kB activity, development of mature B cells, and regulation of immune homeostasis in the skin depended on *ikbkb. ikbkg* is important for liver development.

NF-kB-inducing kinase gene (*nik*) is responsible for lymphoid tissue development (alymphoplasia) and antibody responses.

IkB protein family members participated in embryo- and ontogenesis, inflammation and malignancy. Disorders in *nfkbia* led to embryo lethality, mammary gland carcinogenesis, hyperinflammation, *nfkbib* and *nfkbie* – to impaired cytokine production.

IkB and NF-kB families share two common genes – *nfkb1* and *nfkb2. nfkb1* is important for cytokine production and B cell regulation, *nfkb2* – for lymphoid organs development, antibody production, cytokines expression and bone differentiation. Moreover, *nfkb2* plays crucial role in lymphoproliferative diseases (lymphoma, multiple myeloma, T cell cutaneous lymphoma, B cell chronic lymphocytic leukemia, etc).

Other gene of NF-kB family members is *rela* that encode proteins involved in cell cycle regulation, signal transduction, transcription and metabolism. Its disorders led to lymphoid neoplasia, multiple myeloma and myelination inhibition (possibly, involved in schizophrenia pathogenesis). *crel* had importance for B-cell lymphomas and autoimmune diseases (rheumatoid arthritis, psoriatic arthritis, celiac disease, ulcerative colitis, etc). Impact of *relb* was limited by inflammatory disease (severe acute respiratory syndrome).

Conclusions.

In this review, we retrace some of the important discoveries that have accompanied the description of single IKK-IKB-NF-kB system for both canonical and non-canonical signaling. In particular, we describe gene variability of this system and its phenotypic consequences. IKK-IKB-NF-kB system is important for development/morphogenesis, apoptosis/survival and inflammation/stress. Genetic disorders of this system led to the impair organogenesis and regeneration, malignancy, autoimmune and inflammatory diseases. All members of IKK-IKB-NF-kB system can be the targets for gene investigation and manipulation. Genetic systemic approach can be a powerful tool for the investigation of IKK-IKB-NF-kB system functions and for the discovery of new diagnostic and therapeutic strategies.

Acknowledgements. The author wishes to thank Yulia Lysanets for technical support in manuscript preparation.

Conflicts of interest. The author declares no conflicts of interest.

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Summary

The adaptation of organisms to environmental, mechanical, chemical and microbiological stresses needs inducible regulation of gene expression. One of the most well-known inducible transcription factors is NF-kB. NF-kB has evolutional importance not only for the immune system but also for other organs and systems influencing gene expression that impact cell survival, differentiation, and proliferation. NF-kB signaling mediated by NEMO-dependent or NEMO-independent IKK complexes should be considered within the context of a single regulatory or signaling system. In this review, we retrace some of the important discoveries that have accompanied the description of single IKK-IKB-NF-kB system for both canonical and non-canonical signaling. In particular, we describe gene variability of this system and its phenotypic consequences. Genetic disorders of this system led to the impairment of organogenesis and regeneration, malignancy, autoimmune and inflammatory diseases. All members of IKK-IKB-NF-kB system can be the targets for gene investigation and manipulation. Systemic genetic approach can be a powerful tool for the investigation of IKK-IKB-NF-kB system functions and for the discovery of new diagnostic and therapeutic strategies.

Keywords: NF-kB, polymorphisms, mutation, disease, inflammation, malignancy

Матеріал надійшов до редакції 10.09.2014