

INTERLEUKIN-32 IN INFECTION, INFLAMMATION AND CANCER BIOLOGY

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INTERLEUKIN 32 U INFEKCIJI, INFLAMACIJI I BIOLOGIJI TUMORA

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ABSTRACT

Cytokines are small pleiotropic polypeptides secreted dominantly by the cells of the immune system. These polypeptides are main mediators of innate and acquired immunity, responsible for clonal expansion and differentiation of immune cells, initiation of immune response and enhancing of effector functions of leukocytes. Cytokine-related effects are most studied in the fields of inflammation, immunology, and cancer biology. In this review we discuss one of the most intriguing, recently discovered proinflammatory cytokine, interleukin 32.

Keywords: Interleukin 32, infection, inflammation, tumor

SAŽETAK

Citokini su mali polipeptidi koje luče dominantno ćelije imunskog sistema. Oni su glavni posrednici urođene i stečene imunosti, odgovorni za klonsku ekspanziju i diferencijaciju ćelija imunog sistema, pokretanje imunog odgovora i pojačavanje efektorske funkcije leukocita. Njihovi efekti se najviše izučavaju u oblasti inflamacije, imunologije, i biologije karcinoma. U ovom preglednom radu govorimo o jednom od najinteresantnijih, nedavno otkrivenih proinflamatornih citokina, interleukinu 32.

Ključne reči: Interleukin 32, infekcija, inflamacija, tumor



INTRODUCTION

Cytokines are small proteins secreted by cells of immune system and many others. They are principally involved in homeostatic mechanisms by mediating and regulating inflammatory/immune responses in various diseases and affect cellular interactions and cell communication system (1–4). They direct the development, maturation, localization, interactions, activation and life span of immune cells (2, 5). Cytokines are main mediators of innate and acquired immunity, responsible for clonal expansion and differentiation of immune cells, initiation of immune response and enhancement of effector functions of leukocytes. They are key factors in preventing and stopping an uneventful immune response (1, 3, 6, 7). They also have a role in important physiological processes such as wound repair (3, 5). Almost every biological discipline studies these factors, but cytokine-related effects are uppermost in

the fields of inflammation, immunology, and cancer biology. These peptides act in autocrine, paracrine and endocrine manner, dependent on their site of activity (5). They are principally classified into various groups based on their biological roles. Their characteristic are pleiotropism (activation of numerous types of responses), redundancy (functionally overlapping), synergy (between cytokines to amplify the effect), antagonism (i.e. regulation of duration and potency of the response, important for avoiding autoimmunity), feedback and feedforward loops – for negative and positive (e.g., signal amplification) regulation (3, 5). Cytokines directly influence cancer growth or they indirectly contribute to antitumor activities of lymphocytes (8). In past two decades among great number of discovered cytokines and their functional and regulatory roles, interleukin-32 (IL-32) is one of the most intriguing. Since the



discovery in 1992. it was pronounced as a powerful proinflammatory cytokine with ability to induce production of other proinflammatory cytokines and chemokines (9, 10). The aim of this review is to emphasize actual data and future perspective of interleukin-32 (IL-32).

History of the IL- 32

In 1992. Dahl et al. reported a gene that was highly expressed in activated T- cells and IL-2 activated NK-cells and therefore it was called NK4 (11). This NK4 gene was rapidly upregulated in human peripheral blood mononuclear cells (PBMCs) after stimulation and activation of T-cells. Authors demonstrated high polymorphism of the NK4 gene. Sequence analysis revealed that the NK4 - encoded protein had a predicted molecular mass of 27 kDa (12–14). In addition, it was suggested that the NK4 protein contained Arg–Gly–Asp (RGD) motif, important in cell adhesion (15). The NK4 transcript contains a potential signal sequence cleavage site between amino acid 31 and 32, indicating that NK4 can be secreted by the classical secretion pathway (12, 13). Nevertheless, for the next 13 years, the biological function of NK4 was not known. In 2005, Kim et al. showed for the first time that the NK4 protein had biological function and a recombinant form of the protein induced production of several proinflammatory cytokines (16). It was discovered accidentally while studying the genes induced by IL-18 and was found to stimulate the production of various chemokines, pro-inflammatory cytokines including IL-1 β , IL-6, IL-8, TNF- α and macrophage inflammatory protein-2 (MIP-2) (14, 16). Authors revealed that NK4 protein activates signal transduction pathways such as nuclear factor-kappa (NF-kb) and p38 mitogen activated protein kinase (MAPK). Since the NK4 protein possess significant proinflammatory properties, his name was changed to IL-32.

Isoforms

IL-32 gene was found to be located on human chromosome 16p13.3 and was reported to exist in nine different isoforms by mRNA alternative splicing including IL-32 α , IL- 32 β , IL-32 γ , IL-32 δ , IL-32 ϵ , IL-32 ζ , IL-32 η , IL-32 θ , and IL- 32s (small), and all of them have specific activities and properties (12, 17, 15). IL-32 α is the most abundant while IL-32 β is the most common transcript (12, 18, 19). The different isoforms of IL-32 originate by splicing of pre-mRNA of the isoform IL-32 γ . It was not discovered why the IL-32 γ mRNA transcripts are spliced or is this phenomenon similar in all cells. IL-32 γ is the most potent isoform of IL-32, concerning his role in cell death and cell activation, and this may explain why IL-32 γ is spliced to less harmful isoforms. In addition to promoting cytokine production, overexpression of endogenous IL-32 γ caused cell death, which in contrast doesn't occur with the IL-32 α isoform (15, 20). The difference in the size of the isoforms, ranging from 14.9 kDa (IL-32 α) to 26.7 kDa (IL-32 γ), and the tertiary structure of the isoforms may be part of the explanation for their differ-

ent potency (21, 22). The endogenous level of IL-32 can be modulated in immune cells by exposure to various stimuli. Pathogen-related agents, such as lipopolysaccharide (LPS), muramyl dipeptide (MDP), and double-stranded RNA and several cytokines such as TNF- α and IFN- γ can induce IL-32 expression (23–25). Namely, pathogen-associated molecular patterns (PAMP) and endogenous stress signals termed danger-associated molecular patterns (DAMP) binds to pattern-recognition receptors on cell membrane (26, 27). Exposure of monocytes, macrophages, or endothelial cells to these stimuli induce the expression of endogenous IL-32, both on mRNA and protein levels, via NF- κ B/ activated protein-1 and phosphatidylinositol-3 kinase/Akt signaling pathways. Furhter IL-32 induces various proinflammatory cytokine production via NF- κ B and p38 MAPK (Figure 1). One of the most noteworthy observations is that IL-32 is still not found in rodents, such as mice and rats. Recent discoveries in this area confirmed earlier hypothesis – alternative splicing can be a mighty regulator of isoform types which are produced in various conditions and tissue types (18).

IL-32 signaling and role in cell biology

Earlier investigations concluded that IL-32 downstream signaling involved multiple pathways - NF- κ B and p38 MAPK pathways, Erk1/2 and PI3 K/Akt pathways, and IL-32-exposed human macrophage-like THP-1 cells resulted in the phosphorylation of p300 and DAPK-1 (23, 10, 28, 29). But until today it was not clear whether this cytokine acts on intra- or extracellular level. Further studies revealed high affinity of IL-32 to urinary and neutrophil proteinase 3 (PR3), which consequently (30) proposed as membrane binding protein for IL-32, and regulator of its activity by splicing into the various isoforms (22, 31, 30). IL-32 can also bind to the membrane integrins α V β 3 and α V β 6, but not to α V β 8 (32). Well known fact is that integrins are involved in cell signaling and are important for cell adhesion, survival, and cytokine production. It has been proposed that α V β 3 and α V β 6 could be the receptors for extracellular IL-32 (30). Many reports showed that IL-32 acts inside the cell (30). Releasing of this cytokine is possible after cell death (9, 25). Using special modeling software, some authors conclude that IL-32 has similarities with focal adhesion targeting region (FAT) of focal adhesion kinase (FAK-1) (15). FAT targets FAK-1 to bind with integrin via paxillin. FAK and paxillin are two focal adhesion-associated proteins with crucial function in integrins downstream signaling (33). These signals regulate important biological cell functions, such as migration, proliferation, and survival (34, 35, 33). So far, we can conclude that there are at least two membrane binding proteins for extracellular IL-32 – PR3 and integrins, and another yet unknown receptor (18). Intracellularly, for the activity of this cytokine are responsible FAK-paxillin proteins, which after binding with IL-32 regulate various cell functions, such as cell growth, metabolism, cytokine production, cell adhesion, migration, proliferation, differentiation, angiogenesis and apoptosis (9, 30, 36, 37).

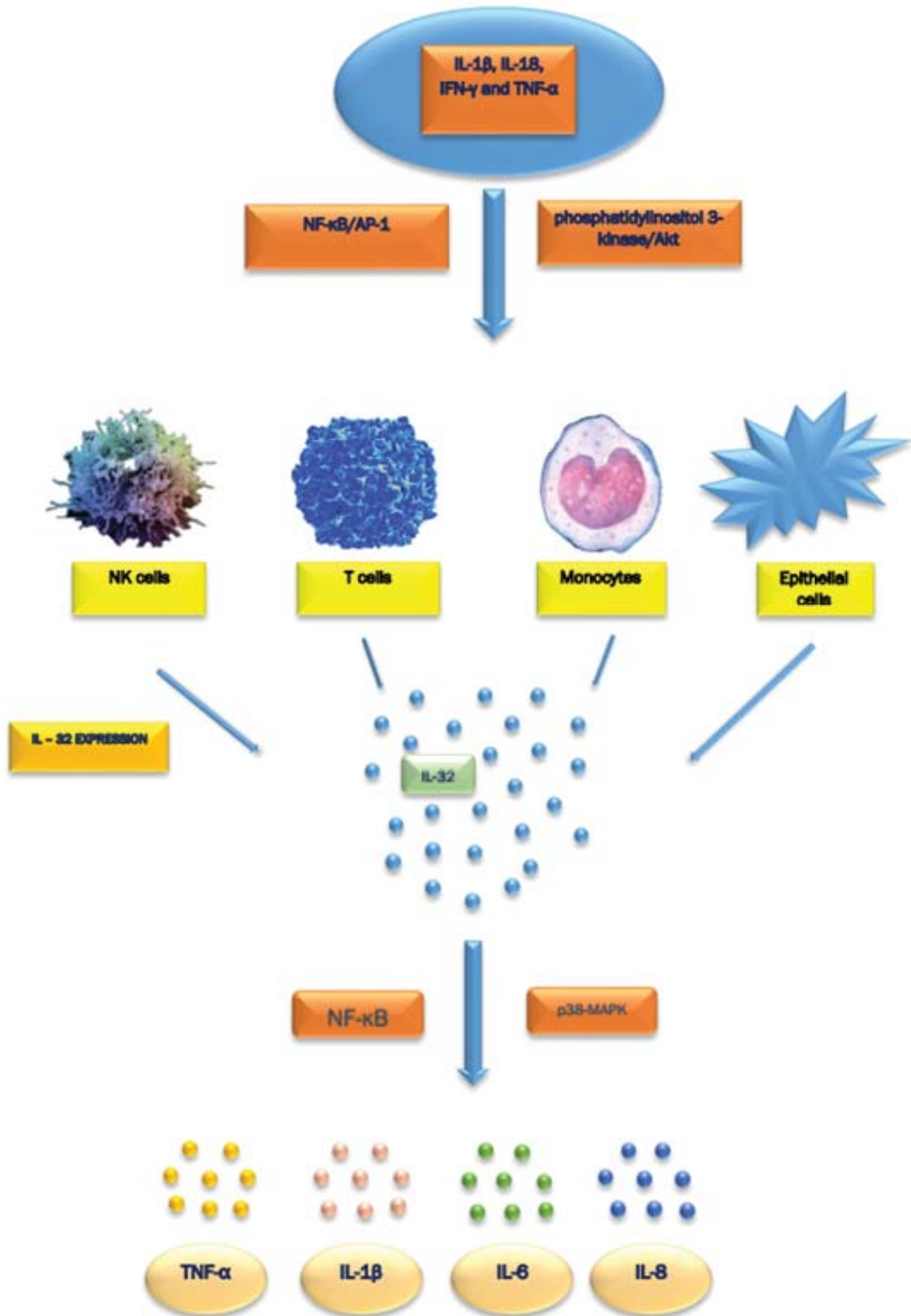


Figure 1. Expression of IL-32 in various cell types upon stimulation with IL-1 β , IL-18, IFN- γ and TNF- α . Signaling pathways are NF- κ B/activated protein-1 and phosphatidylinositol 3-kinase/Akt. IL-32 can induce various cytokines and chemokines - TNF- α , IL-1 β , IL-6 and IL-8 via NF- κ B and p38-MAPK signaling.

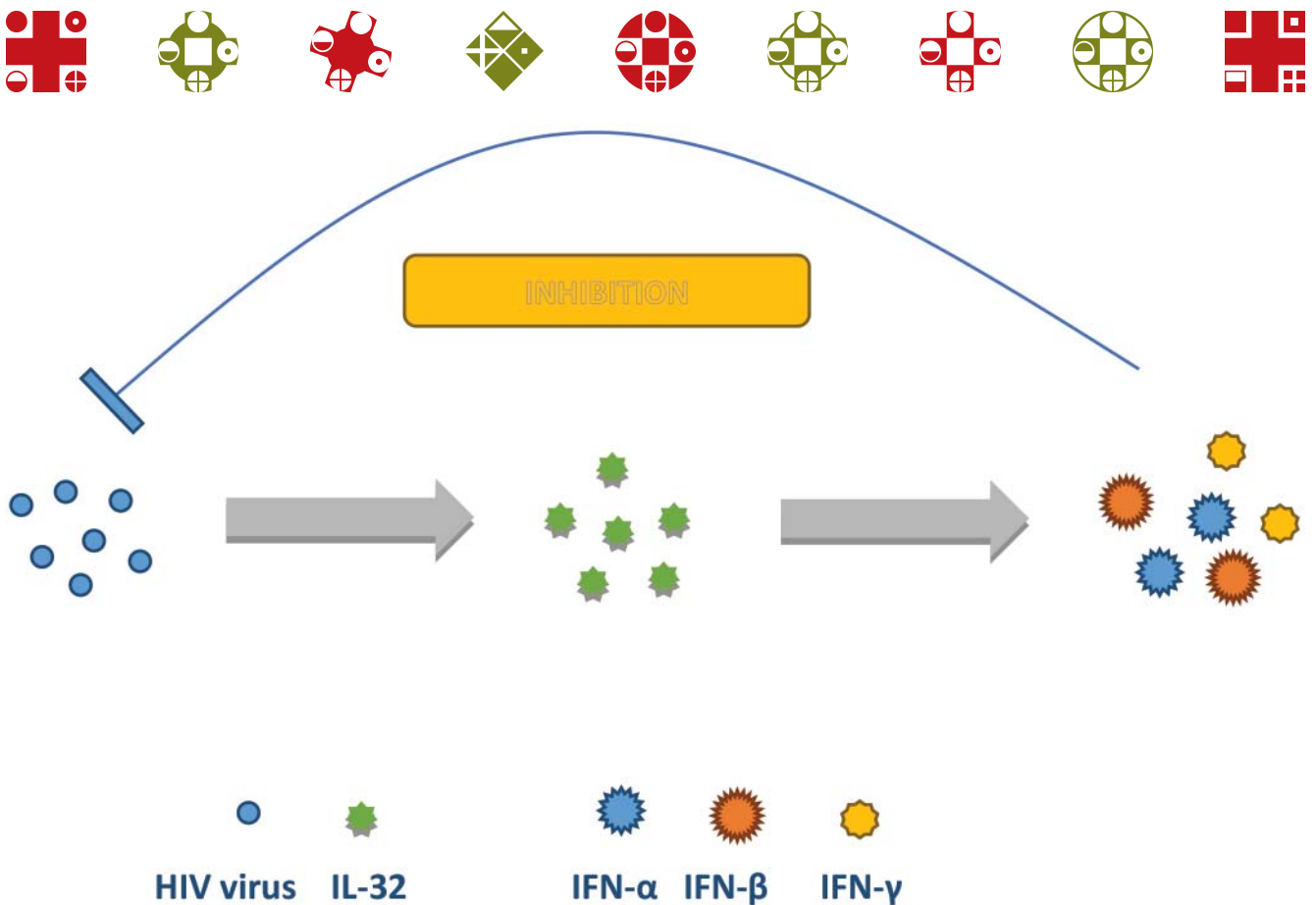


Figure 2. HIV virus induces IL-32 expression. IL-32 promotes induction of various proinflammatory cytokines, and among them most important are interferons - IFN- α , IFN- β , IFN- γ . They suppress further replication of this virus.

Role in viral infections

In last decade many studies published data revealing antiviral IL-32 properties, and increased expression and circulating levels of this cytokine in patients with viral infections. Reports analyzing IL-32 in patients with H1N1 influenza pointed that infected patients had an increased circulating level of IL-32 (38). Antiviral activity of recombinant IL-32 γ was found in WISH (a human amnion cell line) infected with vesicular stomatitis virus (38, 39). Study conveyed earlier revealed that plethora of cytokines were increased in patients with influenza virus: IFN- β , interferons type III, IL 1 α , IL-1 β , IL-6, IL-23, IL-12, and IL-32 γ (14). Influenza A virus induces IL-32 overexpression through NF- κ B and cAMP response element-binding (CREB) pathways (40). Similar studies revealed that IL-32 expression depends on cyclooxygenase-2 (41, 42). Overexpression of IL-32 γ inhibits further replication of this virus. Regarding the role of this cytokine in HIV-1 infections, results showed that interferons are crucial for the anti-HIV-1 effect of recombinant IL-32 γ , and silencing of endogenous IL-32 reduced the levels of Th1 and proinflammatory cytokines, which confirm the anti-HIV-1 property of IL-32. Blockade of any of the interferons α , β or γ enhanced further HIV virus replication (17, 43–45). Based on link between IL-32 activity and production of IFN α , β or γ , various authors proposed that IL-32 exhibits its antiviral properties

by all these interferons (39, 21) (Figure 2). IL-32 expression is induced by hepatitis C and Epstein-Barr viruses (EBV) also (23, 46, 47). Human papillomavirus (HPV) induces IL-32 expression via E7-mediated COX-2 stimulation (41). In hepatitis B virus infection, HBx protein encoded by HBV genome, plays an important role in the hepatic inflammatory processes. Study of Pan X et al. showed that HBx could induce IL-32 expression in Huh7 cell in a dose-dependent manner by NF- κ B pathway (46), and it is correlated with the severity of liver inflammation/fibrosis in patients with chronic HBV infection (48). In vitro and in vivo results of different studies showed that IL-32 expression in human macrophages serves to protect the host by facilitating apoptosis of the host cell and thereby depriving *Mycobacterium tuberculosis* of a protected survival as an intracellular microorganism (49, 50). Similar results were obtained with *Mycobacterium avium* intracellularly. In *M. leprae* infections, form of the disease was dependent of differentiation of dendritic cells induced by IL-32 (51, 52).

Role in inflammatory and autoimmune diseases

Role of IL-32 in rheumatoid arthritis (RA) was intensively studying. Immunohistochemistry staining of synovial tissue specimens revealed expression of this cytokine in sy-



novial lining, sublining, and endothelial cells, especially in macrophage-like cells (53, 54). This expression correlated with inflammation, TNF- α , IL-1 β , IL-18 levels and with acute phase protein CRP and erythrocyte sedimentation rate (ESR) (10, 55, 56). IL-32 was upregulated strongly after stimulation with TNF- α and further induced enhanced production of proinflammatory cytokines IL-6 and IL-8. That could be explanation for the TNF- α /IL-32/TNF- α -positive auto-inflammatory loop and success of the anti-TNF- α therapy in up to 50% of these patients. In another study that enrolled patients with rheumatoid arthritis, osteoarthritis and ankylosing spondylitis serum levels and synovial tissue expression of IL-32 were measured (57). The elevated level of IL-32 γ in ankylosing spondylitis joint positively correlate with osteoblast differentiation via DKK-1 suppression (58). In inflammatory bowel diseases, e.g. ulcerative colitis and Crohn's disease, IL-32 has a important role in pathophysiology and progression. Namely, bacterial peptidoglycan muramyl dipeptide through binding with nucleotide-binding oligomerization domain containing protein 1 (NOD1) and 2 (NOD2) via caspase-1-dependent mechanism leads to induction of IL-32 expression. Further activation of various factors leads to enhanced production of IL-1 β and IL-6, well-known proinflammatory mediators (50, 59). Chronic obstructive pulmonary disease represents inflammatory response to toxic particles and gases. Pathogenesis of this disease involves IL-32, highly expressed in lung tissue specimens, and this expression correlates with degree of airflow obstruction (60, 61). Asthmatic patients had a higher level of systemic IL-32. Studies with this disease pointed that IL-32 inhibits angiogenesis by suppressing VEGF, being endogenous regulator of proangiogenic factors and controller of airway remodeling. It was well documented that IL-32 has an important role in pathogenesis of allergic rhinitis and chronic rhinosinusitis, by stimulating proinflammatory cytokines and chemokines (62, 63). Keratinocytes are a major source of this protein, which enhances their apoptosis and aggravates further worsening in atopic dermatitis. Patients with psoriasis have no significantly increased serum level of IL-32, compared to those with atopic dermatitis (64, 65). In atherosclerosis IL-32 amplifies local inflammation and attracts more circulating monocytes and other inflammatory cells to the subendothelial compartment. Together with activation of matrix metalloproteinases (MMP) 1, 9 and 13, this will contribute to the enhanced vascular inflammation, plaque instability, thickening of the fibrous cap and disruption, leading to acute coronary syndrome (66). Overexpression of IL-32 γ in transgenic mice with provoked sepsis lead to more severe disease (67).

Role in cancer biology

Currently there are very actual reports about role of this cytokine in cancer and cancer therapy. One of the first that imposed possible role in cancer explained that in chronic myelomonocytic leukemia IL-32 expression was markedly reduced, while in myelodiplastic syndrome was elevated

and associated with enhanced apoptosis of the bone marrow stem cells (68, 69). Several reports confirmed that IL-32 was highly expressed in tumor tissue, compared to adjacent tissue without cancer cells: brain, breast, lung and stomach cancer (30). In vitro studies showed that this protein induces migration and invasion of cancer cells. Overexpression of IL-32 contributes to invasion and metastasis in primary lung adenocarcinoma, induced by increased expression of MMPs 2 and 9 via NF- κ B activation (70). IL-32 α exhibits more migratory ability to melanoma cells, through downregulation of E-cadherin expression (71). In stomach cancer, this cytokine is involved in process of carcinogenesis since beginning. That was proved in patients with confirmed *Helicobacter pylori* inflammation, which is confirmed carcinogen (72, 73). Their gastric mucosa expresses higher levels of IL-32, as well as levels in sera of same patients. Opposite to these findings, there are several reports about inhibitory effects of IL-32 on cancer cell growth, especially via the NF- κ B and STAT3 signaling (29). IL-32 θ inhibited epithelial-mesenchymal transition (EMT), resulting in the suppression of migratory and invasive capabilities of HT29 colon cancer cells (74). Higher levels of IL-32 were reported in many other cancers: melanoma, thyroid, renal cell (18, 71, 75). Recent studies have revealed higher expression of IL-32 in human pancreas, liver, and esophagus cancer tissues, compared with normal tissue or serum (76–78). One of the hallmarks of tumor is angiogenesis. Data about role of IL-32 in this process are still controversial and insufficient. Examination of the in vitro and in vivo models on endothelial cells (EC) in pulmonary arterial hypertension- PAH and glioblastoma multiforme (GBM) showed that IL-32 requires cofactor (IFN- γ) to sensitize EC, i.e. to exert its biological activity (32). Also, examination of effects in neonatal HUVEC (human umbilical vein endothelial cells) and adult pulmonary microvascular EC, using an in vivo and an in vitro angiogenesis assay, showed that induction of IL-32 in ECs was related with activation and proliferation of these cells, as well as angiogenesis (32). Angiogenic effect in certain concentrations on HUVEC was even greater than with VEGF, but was not dependent on VEGF(18, 32). Authors conclude that IL-32 exerts its angiogenic properties in EC via integrins (at least in some part), requires second stimuli (with LPS, IFN- γ or unknown cofactor) for endothelial cell responsiveness. IL-32 utilize regulation of IL-8, MMP-9, activin A, and endostatin, but not VEGF or TGF- β 1 to induce angiogenesis in EC (32). In other study with asthmatic patients, assays with normal human bronchial cells stimulated with TNF- α , IFN- γ , Th1 cells and rhinovirus infection were examined(79). Results showed that IL-32 inhibited angiogenesis by decrease of VEGF production in these cells. Authors conclude that the IL-32-mediated decrease in VEGF secretion by NHBE cells during airway inflammation supports the anti-angiogenic effect of this cytokine (79). Interestingly, there are no literature data about possible role of IL-32 in lymphangiogenesis and consequently role in spread of cancer through the lymphatic system.



Conclusion and future perspectives

IL-32 and its modulatory role in innate and acquired immunity and other processes are still under intense investigation. From known facts it could be concluded that main biological functions of IL-32, acting predominantly intracellularly, include induction of the expression of various pro-inflammatory and some anti-inflammatory cytokines and, hence, contribution to the progression and pathogenesis of various pathological conditions and systemic infections. Important issue and role in tumor angiogenic processes and carcinogenesis should be explored. That could influence and modify further therapeutic strategies in various pathological conditions.

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Conflict of interest

The authors declare no financial or commercial conflict of interest.

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