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ABSTRACT

Interleukin-32 (IL-32) was originally identified in natural killer (NK) cells activated by IL-2 in 1992. Thus, it was named NK cell transcript 4 (NK4) because of its unknown function at that time. The function of IL-32 has been elucidated over the last decade. IL-32 is primarily considered to be a booster of inflammatory reactions because it is induced by pro-inflammatory cytokines and stimulates the production of those cytokines and *vice versa*. Therefore, many studies have been devoted to studying the roles of IL-32 in inflammation-associated cancers, including gastric, colon cancer, and hepatocellular carcinoma. At the same time, roles of IL-32 have also been discovered in other cancers. Collectively, IL-32 fosters the tumor progression by nuclear factor-κB (NF-κB)-mediated cytokines and metalloproteinase production, as well as stimulation of differentiation into immunosuppressive cell types in some cancer types. However, it is also able to induce tumor cell apoptosis and enhance NK and cytotoxic T cell sensitivity in other cancer types. In this review, we will address the function of each IL-32 isoform in different cancer types studied to date, and suggest further strategies to comprehensively elucidate the roles of IL-32 in a context-dependent manner.

INTRODUCTION

Interleukin 32 (IL-32) was initially identified in activated natural killer (NK) and T cells, and its expression is strongly augmented by microbes, mitogens, and other pro-inflammatory cytokines. Accumulating evidences has shown that IL-32 is an amplifier of inflammation through its stimulatory effects on pro-inflammatory cytokines, including IL-1β, IL-6, and tumor necrosis factor α (TNF-α). Thus, it is likely that prolonged or over-activation of IL-32 would play a role in some chronic inflammation-related diseases, including rheumatoid arthritis (RA), Crohn's disease, and cancer. Indeed, since many studies have uncovered the molecular mechanisms by which IL-32 exerts anti-tumor or pro-tumor effects, it is appropriate to summarize the detailed functions of IL-32 in various cancers to understand a precise role of IL-32, and to present the direction for future research. Thus, we will review the effects of IL-32 on various cancers in three aspects: first, a role of IL-32 in tumors which are highly related to inflammation; second, a role of IL-32 in tumors which are not related to inflammation; and lastly, effects and functions of IL-32 in pro- or anti-tumor immune cells.

1. Roles of IL-32 in inflammatory cancers

Development of malignant tumors follows a dynamic sequential progression that includes initiation, malignant conversion, and metastasis. As tumor mass increases progressively, tumor cells need to communicate with components of the tumor microenvironment for maintenance of tumor mass, consisting of infiltrating immune cells and stromal cells. Interactions between tumor and immune cells in tumor development are also crucial factors of the tumor microenvironment (1). Therefore, tumor infiltrating immune cells are considered accurate independent prognostic factors, superior to tumor node metastases (TNM) tumor stage (2). The most common subsets of tumor infiltrating lymphocytes (TILs) are clusters of $CD3^+$, $CD4^+$, $CD8^+$, and Forkhead box $P3^+$ ($Foxp3^+$) T lymphocytes. $CD4^+$ T lymphocytes were classified into Th_1 and Th_2 cells in the early 1980s, based on different T cell functions (3). Th_1 cells promote the toxic effects of cytotoxic T lymphocytes (CTLs) and Th_2 cells enhance the antibody-mediated humoral immune response. Recently, $CD4^+Foxp3^+$-expressing T lymphocytes were identified as regulatory T lymphocytes (Treg). Tregs play a role in suppressing the immune system and promoting cancer progression (4). Therefore, it is conceivable that several cancers are caused by chronic infection and inflammation, after acquiring various harmful genetic alterations**.** Chronic inflammation of the esophagus can increase incidence of esophageal carcinoma (5). Cirrhosis and inflammation of the liver can develop into hepatocellular carcinoma (6). Chronic pancreatitis increases the incidence rate of pancreatic cancer (7). Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC) have been linked to the development of colon adenocarcinoma (8). Since IL-32 expression is increased in inflammatory diseases, such

as IBD and RA, and amplifies inflammatory cytokines, it is conceivable that IL-32 is strongly associated with various cancers related to inflammation.

1.1 Gastric cancer (GC)

Even though most infected individuals remain asymptomatic throughout their life (9), infection with *Helicobacter pylori* (*H. pylori)* causes chronic gastric inflammation. Approximately 10% of *H.pylori*-infected individuals have been reported to develop peptic ulcer disease and 1 to 3% of them progress to GC (10). IL-32 expression in sera from 16 GC patients was first detected using an IL-32 enzyme linked immunosorbent assay (ELISA), and IL-32 was slightly increased compared to 10 healthy donors. IL-32 expression was highly increased in the gastric mucosa from GC patients, while it was rare in healthy gastric mucosa (11, 12), but the increased amount of IL-32 was mainly localized in the cytoplasm of gastric epithelial cells from gastritis and GC patients (11).

Several isoforms of IL-32 can be generated through alternative splicing (13, 14). The complete transcript that contains all exons is IL-32γ. IL-32γ mRNA can be spliced to produce shorter isoforms, including IL-32α, IL-32β, and IL-32δ (15-17). Other isoforms of IL-32 have not been well-studied yet. These isoforms show context-dependent function. For example, IL-32γ and IL-32β have been shown to induce caspase-8-dependent apoptosis in thyroid cancers (16), but not in breast cancers (18). Human gastritis tissue, as well as a gastric cancer cell line AGS cells expressed mostly IL-32β and marginally IL-32ε, without detection of other isoforms (11). *H. pylori* infection increases in the cytoplasmic IL-32 expression in AGS cells were found to be dependent on the bacterial cytotoxin-associated gene pathogenicity island (cagPAI)

genes, a cluster of approximately 30 genes, which critically influences the progression of GC. The cagPAI-mediated IL-32 production depended on nuclear factor kappa B (NF-κB) activation (11). However, phosphorylation of NF-κB was not observed in AGS cells treated with recombinant IL-32β. Thus, IL-32β is considered to have a specific intracellular function in GC tissue or GC-derived immortal cells, instead of a paracrine function (11). Indeed, *H. pylori* infection-induced IL-8, C-X-C motif chemokine 1 (CXCL1), CXCL2, and TNF-α production was impaired in IL-32 deficient AGS cells (11). On the other hand, a single nucleotide polymorphism (SNP) in the IL-8 gene, which is associated with increased IL-8 expression, was identified as a risk factor for GC in the Japanese population (19). Considering that IL-32β increases IL-8 production, it is a possible that IL-32β overexpression in GC may be associated with GC carcinogenesis. Wang et al. performed genetic analyses and found that a SNP in IL-32β itself was associated with GC carcinogenesis. The study showed a positive relationship between a SNP on IL-32 rs2015620 in the Chinese population and GC (20).

In addition to the possibility that IL-32β is associated with GC carcinogenesis, the role of IL-32 in GC progression and metastasis was also revealed by analysis of 120 patients diagnosed with GC (21). Cytoplasmic IL-32 expression was stronger in malignant stages of GC, and IL-32β was the major isoform found, with minor expressions of IL-32α and IL-32γ. Moreover, both IL-32 isoforms were highly expressed in patients with invaded serosal surface of the gastric walls and lymph node metastasis. This study supports the possibility that IL-32 could be a prognostic marker for GC. The underlying molecular mechanism of IL-32 on GC invasion has also been elucidated. Human GC TSGH9201 cells overexpressing IL-32γ showed increases in

both intra- and extracellular levels of IL-32β, and augmented migration and invasion capacities. Furthermore, increased activation of AKT-β-catenin signaling pathway by IL-32β was accompanied by increased production of IL-8, vascular endothelial growth factor (VEGF), and active matrix metalloproteinase 2 and 9 (MMP2 and 9) (15). Clarification is needed regarding whether this IL-32β-mediated GC invasion is mediated by intra- or extracellular IL-32 or both. Although several studies showed the positive relationship between expression levels of IL-32 and metastasis and invasion of GC in patients (21, 22), one study showed that IL-32 had a non-significant relationship with the GC malignancy marker TNF-α in patients with GC (23). This discrepancy suggests that experiments with a larger GC patient population are needed in future studies.

In general, tumor infiltrating immunosuppressive cells, including tumor associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and Tregs are correlated with poor GC prognosis, whereas immune activator and effector cells, including CTL, dendritic cells (DCs), and CD45RO memory T cells are associated with an improved GC prognosis. Those immune cells affect the progression and malignancy of GC as they produce and secrete various immunoregulatory soluble factors into the tumor microenvironment. Chang et al. reported that high levels of IL-32, along with IL-6, IL-10, C-C motif chemokine ligands (CCL) 7, and CCL21 were observed in the sera of GC patients and were associated with poor prognosis (24). Although functions of extracellular and circulating IL-32 in GC cells were not investigated in that study, it is a quite possible that circulating IL-32 performs a functional role in immune cells in the tumor microenvironment, considering that circulating IL-32 positively regulates pro-inflammatory cytokines, including IL-1, IL-6, and TNF-α.

Collectively, *H. pylori* infection-induced IL-32β was the major isoform found in GC and the resultant IL-32β stimulated the production of various cytokines, including IL-8, CXCL1, CCL21, MMP2/9, and TNF-α. These inflammatory cytokines would be associated with GC carcinogenesis and invasion. However, extracellular IL-32β did not affect cytokine production in AGS cells (11). Therefore, further studies are needed to determine whether extracellular IL-32β could affect the properties of GC cells using various GC cell lines.

1.2. Pancreatic cancer

IL-32 is more highly expressed in inflamed lesions of chronic pancreas, compared to those in normal pancreatic duct cells. In addition, a strong expression of IL-32 has been observed in pancreatic cancer tissues. Pro-inflammatory cytokines IL-1, interferon γ (IFN-γ), and TNF-α stimulated IL-32 production in pancreatic cancer cell lines, including PANC-1, MIA PaCa-2, and BxPC-3 cells, which weakly express IL-32 without inflammatory stimulation. Activation of the phosphoinositide 3-kinase (PI3K)-Akt signaling pathway was required to induce IL-32 expression by those cytokines. Inhibition of NF-κB and activated protein-1 (AP-1) signaling pathways markedly suppressed the IL-1, IFN-γ, and/or TNF-α-induced IL-32 mRNA expression (25). Collectively, IL-32 expression was induced by PI3K-Akt signaling pathway-dependent NF-κB-AP-1 signaling activation. Since small interfering IL-32 RNA (siIL-32) reduced pro-survival proteins (B cell lymphoma 2, Bcl-2; B cell lymphoma extra large, Bcl-xL; and myeloid cell leukemia sequence 1 protein, Mcl-1) without changing pro-apoptotic proteins (Bcl-2 associated X protein, Bax; Bcl-2 antagonist killer 1, Bak; BH3

interacting domain death agonist, Bid; Bcl-2 associated death promoter, Bad), IL-32 is likely to promote growth and survival of pancreatic cancer cells. On the other hand, a functional isoform of IL-32 in pancreatic cancer cell has not been reported. Therefore, further studies are needed to determine the isoform of IL-32 responsible for pro-survival effects in pancreatic cancer cells.

1.3. Colon cancer

The risk of colorectal cancer (CRC) is positively proportional to the extent and duration of IBD, such as UC and CD (26). Since IL-32 is a pro-inflammatory cytokine and CRC is one of well-known inflammation-induced cancers, the effect of IL-32 on CRC was determined in an azoxymethane (AOM)-induced CRC model using human IL-32α transgenic (TG) mice. The expression of IL-32α showed protective effects on AOM-induced CRC incidence (27). The underlying mechanism was reported that the induction of TNF receptor 1 (TNFR1) expression by IL-32α in tumors, which causes apoptosis of tumor cells. Even though the IL-32 gene is not found in rodents, human IL-32α seems to be able to induce the apoptotic death of mouse tumor cells. This result suggests a role for human IL-32 in the mouse cells. The study also confirmed that TNF-α induced cell death in IL-32α-expressing human SW620 colon cancer cells. IL-32α induced sustained c-Jun N-terminal kinases (JNK) activation via reactive oxygen species (ROS) production, resulting in the apoptotic death of SW620 cells. A relationship between IL-32α and TNFR1 has also been suggested from studies of tumor tissue from CRC patients, where IL-32α and TNFR1 were co-expressed. Expression of IL-32α and TNFR1 increased until CRC reached stage II, and decreased in stage III and

IV. This suggests that IL-32α exerts suppression of CRC growth at the initial stage of tumor progression. On the other hand, why and how IL-32α expression decreases as CRC stage progresses remains to be investigated. In a study by Yun et al., a single injection of AOM induced CRC, without subsequent dextran sodium sulfate (DSS) treatment. Since AOM is a genotoxic chemical and used for initiation of genomic mutations, a single intraperitoneal injection of AOM is usually followed by a 1-week exposure to 2% DSS in the drinking water, and results in a 100% incidence of colonic adenocarcinoma (28). Therefore, the protective effect of IL-32α against CRC growth is not likely due to an inflammatory mechanism, because the mice were not co-treated with DSS, which is a non-genotoxic chemical that induces a reproducible inflammation (29). Therefore, the AOM-DSS-induced CRC model can provide answers on how the inflammatory function of IL-32α affects tumor growth, and which inflammatory cells are recruited into the tumor mass. Contrary to the study of Yun et al., Yang et al. investigated whether IL-32 expression has clinical significance in CRC metastases using a total of 70 CRC patients, including 47 cases of single CRC organic metastasis lesions. They found that IL-32 expression increased in the organic metastasis and the lymph node metastasis of CRC. Thus, IL-32 expression is likely to stimulate the organic metastasis and the lymph node metastasis of CRC (30). Interestingly, recent reports have suggested that IL-32θ would have anti-CRC effects through its anti-inflammatory function (31), while others have shown it to be a CRC inducer under obesity-induced inflammation conditions (32). To resolve this discrepancy, further analysis on larger populations is required and it should be determined which IL-32 isoform is involved in metastases of human CRC patients.

1.4.Hepatocellular carcinoma

Hepatic fibrosis is initiated upon hepatocyte damage, followed by the recruitment of inflammatory cells and can further progress to chronic liver diseases and cirrhosis, and even to hepatocellular carcinoma (HCC) (33). Hepatic fibrosis is characterized by the progressive accumulation of extracellular matrix (ECM) secreted by hepatic stellate cells (HSCs), and the balance between ECM production and degradation, mediated by matrix metalloproteinases (MMPs), and endogenous inhibitors of metalloproteinases (TIMPs). IL-32 induces cytokines, including IL-1β, IL-6, IL-8, and TNF-α. (34, 35), which are highly increased in the liver with severe fibrosis (34, 36, 37). Moreover, recombinant IL-32γ treatment of LX-2 cells, an ECM-producing human hepatic stellate cell line, was enough to induce TIMP-1 expression by activation of the AP-1 signaling pathway (34). Moreover, the increase of TIMP-1 expression promoted the migration of LX-2 cells. In that study, the authors suggested that IL-32 might be a new therapeutic target for hepatic fibrosis as well as hepatitis, because hepatitis B virus (HBV) and hepatitis C virus (HCV) infections could induce IL-32 expression, as well as liver fibrosis in hepatocytes (38, 39).

IL-32α levels in the tumor region from patients with HCC were markedly higher than in the non-tumor region, and HCC patients also showed high levels of circulating IL-32α levels (40, 41). In tumor tissue, IL-32α localized in the cytoplasm and IL-32-expressing tumor cells were located at the invasion site of blood vessels. IL-32α stimulated cell survival and growth through NF-κB activation and maintenance of the Bcl-2 anti-apoptotic protein in the SK-Hep1, HCC cell line. Additionally, tumor

promoting activity of IL-32α was confirmed using an HCC xenograft mouse model with various cell lines. One study investigated whether IL-32 would be an accurate marker for early diagnosis of HCC using 50 HCC patients and 15 healthy volunteers (42). The mean serum level of IL-32 was higher in patients with HCC than in the control subjects ($p<0.001$), but did not correlate with survival rate. Those experimental data support IL-32 as a prospective diagnostic marker for HCC along with alpha-fetoprotein (AFT) and IL-18, but not as a prognostic marker. Further studies are needed to determine the levels of IL-32 in the sera of patients with HCC, cirrhosis, and stage-dependent HCC patients to validate the possible application of IL-32 as an early diagnosis marker for HCC. Recently, Kim et al. reported that IL-32γ showed anti-viral effects against HBV. Intracellular IL-32γ inhibited the transcription and replication of HBV through non-cytopathic pathway in hepatocytes (43). Therefore, further investigation of this novel effect of IL-32γ on HBV clearance in the carcinogenesis of hepatocytes is needed.

1.5 Lung Cancer

Initiation and progression in lung cancer, as in other cancers, are primarily driven by genetic alterations (44) and are strongly linked to inflammation. IL-32 expression is highly up-regulated in most adenocarcinomas, but not in squamous-cell carcinomas (SCC). IL-32–expressing TILs, mainly composed of $CD68^+$ macrophages, $CD4^+$ T lymphocytes, and dendritic cell (DC)-specific intracellular adhesion molecule-3-grabbing non-integrin DC ($DC\text{-}SIGN^+$), and IL-32-expressing tumor cells are frequently observed in lymph node metastases, along with IL-6, IL-8, and VEGF (45, 46). Thus, it is likely that IL-32 significantly correlates with lung tumor progression stages, lymph

node and distant metastases with poor prognosis (45). IL-32 expression is associated with acquisition of an invasive and metastatic phenotype of lung cancers. As an underlying mechanism, amplification of the loop of IL-32 and NF-κB is related with enhanced migration via up-regulation of MMP2/9. On the other hand, IL-32 polymorphisms (rs12934561 and rs28372698) are closely associated with poor survival in SCC, and with poor prognosis in moderate and well-differentiated lung cancer patients (47). On the other hand, Yun et al. recently reported on the tumor-suppressive effect of IL-32γ in the lung (48). In this study, mice overexpressing IL-32γ showed a suppressive effect on growth of carcinogen-induced lung tumors with increased TIMP-3 and reduced NF-κB activity. Additionally, expression levels of both IL-32 and TIMP-3 were decreased in the lung cancer tissues compared to normal lung tissues. These discrepant roles of IL-32 in the pathogenesis of lung cancer are presumed to be due to the different reactivity of IL-32 to various carcinogens, as well as the different roles of extra- and intracellular IL-32. This discrepancy should be further elucidated. The up/downstream signaling pathways of IL-32 in inflammatory cancers are summarized in Figure 1.

2. Role of IL-32 in other types of cancer

2.1 Women's cancer

2.1.1. Breast cancer

We previously reported that IL-32β and IL-32γ mRNAs were highly increased in MDA-MB-231 malignant breast cancer cells, but only that IL-32β protein was detected by Western blotting (49), consistent with a previous report showing that IL-32γ mRNA splices into IL-32β, followed by mainly expression of IL-32β protein (15). The expression level of IL-32β was positively correlated with tumor volume, metastasis to lymph nodes, and tumor stage. MDA-MB-231 cells expressing IL-32β exhibited increased migration and invasion capacities, with increases in epithelial mesenchymal transition (EMT) markers, including vimentin and slug. As an underlying signal transduction mechanism, we showed that IL-32β enhanced migration and invasion through increase of soluble factor VEGF production by signal transducer and activator of transcription 3 (STAT3) activation. Later, Wang et al. also showed that tumor growth was increased with administration of recombinant IL-32 protein to MDA-MB-231 tumor-bearing mice, and that IL-32 increased proliferation of MCF-7 cells and inhibited apoptosis induced by serum starvation (18). On the other hand, since IL-32 enhances VEGF production under hypoxic conditions in breast cancer, it is conceivable that IL-32β is regulated by hypoxia. Indeed hypoxia induces IL-32β production through ROS production (50), which was proven by showing that IL-32β production was suppressed by both nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and

mitochondrial ROS inhibitors. Under normoxic conditions, IL-32β interacts with the Von Hippel-Lindau (VHL) E3 ligase complex, independent of hydroxylation of IL-32β. Thus IL-32 is degraded through ubiquitination. In hypoxic-conditioned cells, hypoxia-induced ROS disrupts the interaction between VHL and IL-32β, leading to accumulation of IL-32β (50).

The pro-inflammatory cytokines IL-1β and IL-18 are important amplifiers of the inflammatory process with IL-32. These cytokines are produced as pro-IL-1β and pro-IL-18 and inflammasomes facilitate maturation of pro-IL-1β and pro-IL-18 under infection and tissue damage conditions. Without inflammasome signals, pro-IL-1β is degraded through ubiquitination. Upon generation of an activation signal, it is de-ubiquitinated and processed into its mature form (51). IL-32β is another interleukin which is regulated by ubiquitination.

On the other hand, hypoxia-induced IL-32β interacts with protein kinase C δ (PKCδ) and this interaction results in the suppression of PKCδ-induced apoptosis (52). We also showed that the accumulated IL-32β translocates to the mitochondria under hypoxic conditions. To investigate the role of mitochondrial IL-32β, metabolic alteration was examined. The hypoxia-induced IL-32β enhanced glycolysis through activation of lactate dehydrogenase through the sustained activation of proto-oncogene tyrosine-protein kinase Src (50). This was the first report showing that IL-32β plays a role in the regulation of breast cancer cell metabolism. A recent report suggested that IL-32 is a prospective therapeutic target for triple negative breast cancer, since IL-32 was more highly expressed in cancer tissue from patients with triple negative breast cancer (53), implying a critical role of IL-32 in the progression of the breast cancer.

2.1.2. Cervical cancer

IL-32 was highly expressed in tissues showing classical morphological features of human papillomavirus (HPV) infection, including koilocytosis, acanthosis, and papillomatosis from cervical cancer patients, but not in tissues lacking HPV-associated nuclear atypia. However, IL-32 expression did not correlate with survival rates of cervical cancer patients. When HPV E7 oncoprotein was induced in cervical cancer cells, such as SiHa and C33A cells, IL-32 expression was induced by cyclooxygenase 2 (COX2), which is an enzyme producing inflammatory mediators, and suppression of COX2 failed to induce IL-32 by HPV E7 oncoprotein (54). Interestingly, IL-32γ overexpression inhibited E7 oncoprotein and COX2 expressions in SiHa and CaSki cells. These results imply that E7-induced IL-32γ suppresses E7 and COX2 expression by a negative feedback loop. In addition, Lee et al. showed that IL-32 overexpression increased the levels of p21 and cleaved-poly ADP-ribose polymerase (PARP), as well as the suppression of cyclin E and cyclin A expression. Thus, HPV infection-induced IL-32 may contribute to the inhibition of tumorigenesis in cervical cancer cells (55). On the other hand, IL-32β expression was increased in T cells undergoing activation-induced cell death, and inducible expression of IL-32 in a HeLa human cervical cancer cell line caused apoptosis, while IL-32 knockdown inhibited apoptosis (56). In addition, phospholipase A2-activating protein (PLAA) enhanced cisplatin-induced apoptosis in HeLa cells and cisplatin induced IL-32 expression in cells expressing high PLAA (57). Contrary to its role in GC, IL-32β is involved in the apoptosis of cervical cancer.

2.2 Lymphoma

2.2.1. Gastric B-cell lymphoma

IL-32 and COX2 expressions were immunohistochemically detected in 31 primary gastric B-cell lymphoma patients and 19 chronic gastritis patients, and were significantly higher in *H. pylori*$^{+}$ lymphoma tissues compared with *H. pylori*$^{-}$ lymphoma tissues (58). This is reasonable because an increase in COX2 in the human stomach is associated with *H. pylori* infection (59, 60) and COX2 increases IL-32 expression (61). Cui et al. also showed (58) that IL-32 and COX2 expressions were significantly correlated with greater *H. pylori* infection, more frequent lymph node metastasis, and advanced stages of gastric B-cell lymphoma. In addition, patients with higher COX2 and IL-32 levels showed a poorer prognosis compared with those patients with lower COX2 and IL-32 levels, but the difference was not statistically significant. On the contrary, high levels of IL-32 could inhibit HPV-induced tumorigenesis, and increases in COX2 and IL-32 seem to be associated with poor prognosis of gastric B-cell lymphoma. This is another example showing that IL-32 exerts context-dependent function.

2.2.2 Cutaneous T-cell lymphoma (CTCL)

Cutaneous T-cell lymphoma (CTCL) is characterized by clonal expansion of malignant T-cells, and the most common type of CTCL is mycosis fungoides (MF). MF progresses from flat erythematous patches to the tumor stage by expansion of malignant T-cells. An *in silico* approach involving meta-analysis revealed specific genes for MF tumor

stages, which were expressed at significantly higher levels in MF and showed that MF originates from IL-32-producing cells (62). In addition, Suga et al. showed that serum IL-32 levels were positively correlated with disease activity within individual CTCL patients. Keratinocytes express IL-32 in the skin of MF patch and plaque, whereas in MF tumor, atypical T cells do. Thus it is clear that both tumor and stromal cells express IL-32. A study also revealed that IL-32 stimulated proliferation of a MF cell line, MyLa and Sezary syndrome cell line, and SeAx cells through mitogen-activated protein kinase (MAPK) and NF-κB activation, without inducing apoptosis (63). Since the addition of anti-IL-32 antibodies to culture inhibited the proliferation of SeAx cells and the viability of MF cells, it is likely that IL-32 serves as an autocrine, as well as paracrine cytokine in CTCL. This study clearly shows that circulating IL-32 can directly affect tumor cells. The up/downstream signaling pathways of IL-32 in non-inflammatory cancers are summarized in Figure 2.

3. Effect of IL-32 on cancer-related immune cells

3.1. IL-32 enhances NK cell sensitivity and cytotoxicity against tumor cells

IL-32 was originally identified as a cytokine produced and secreted from NK cells and NK cells are well known innate immune cells which display tumor-killing capacity. Thus, it is conceivable that IL-32 affects tumor growth by modulating NK cell activity. Indeed, Cheon et al. reported that IL-32α overexpressing chronic myeloid leukemia (CML) cells, including K562, Kcl22, and BV173, were highly susceptible to NK cell-mediated killing. This enhanced susceptibility to NK cells was due to IL-32α-stimulated increases in Fas and UL16-binding protein 2 (ULBP2) via activation of p38 MAPK in CML cells (64). Both Fas and ULBP2 are key players associated with NK cell cytolytic activity. NK cells expressing Fas ligand (FASL) effectively eliminate tumors expressing Fas. ULBPs are the most typical ligands of the natural killer group 2D (NKG2D), which is a critical activating receptor on the surface of activated NK cells (65, 66). Thus, tumor cells expressing ULBPs are susceptible to activated NK cells. Further study are needed to determine whether IL-32α can also induce death signal receptors in other cancer types. In addition to the effect of IL-32α on enhancement of tumor cell sensitivity against NK cells, Park et al. showed that IL-32 also enhanced NK cell cytotoxicity (67). Co-incubation of a human NK cell line NK-92 cells with PC3 prostate cancer cells or SW620 colon cancer cells showed a significant inhibition of tumor cell growth. The death receptor TNFR2 and death receptor 3 (DR3) were upregulated in PC3 cells, and both FAS and DR3 were also increased in SW620 cells by co-culture of PC3 cells with NK-92 cells, leading to susceptibility to NK-mediated killing. However,

IL-32 deficient NK-92 cells failed to induce expression of death receptor on tumor cells. It is known that TNF-related apoptosis-inducing ligand (TRAIL), TNF, FASL, and DR3 ligand (also known as APO3L) are released by NK-92 cells and induce cancer cell death (68, 69). Thus, Park et al. showed that expression of DR3 ligand in NK cells was upregulated in NK-92 cells after co-culture of NK cells with cancer cells, and inhibited by IL-32 siRNA treatment (67). Additionally, NK cells eliminated the tumor cells through cytotoxic perforin/granzyme-containing granule exocytosis and the nitric oxide (NO), but IL-32 did not affect this pathway. How IL-32 is involved in increasing the DR3 ligand on NK cells and its death receptor on tumor cells should be the topic of future studies. Collectively, IL-32 affected NK cell cytotoxicity and susceptibility of tumor cells to NK cells (Figure 3A).

3.2 IL-32 recruits CTLs and NK cells into tumor mass

To determine a systemic role of IL-32, Yun et al. generated IL-32β TG mice in which circulating IL-32 levels were increased (70). Interestingly, IL-32β TG mice showed attenuated growth of subcutaneously implanted B16 melanoma with increased pro-apoptotic genes. This suggests circulating IL-32β directly induced apoptosis of B16 melanoma cells. Using a CRC xenograft model of SW620 cells and prostate cancer cells (PC3) expressing IL-32β, tumor growth and mass were reduced, and the number of CTLs and NK cells in blood, spleen, and solid tumor was increased. This implies that IL-32β could play a role in the tumor microenvironment.

Oh et al. also generated IL-32γ TG mice and observed that B16 melanoma growth was suppressed in IL-32γ TG mice. in addition, IL-32γ-overexpressing colon cancer

cells showed inhibition of tumor growth in a xenograft model. The inhibitory effect of IL-32γ on tumor growth was associated with the suppression of constitutively activated NF-κB and STAT3 in tumor tissues and in colon cancer cells (71). The authors also investigated whether the inhibition of colon cancer growth by IL-32γ was related to tumor-specific immune responses. A significantly higher number of CTLs, as well as NK cells was observed in the tumor tissues. Both IL-32β and IL-32γ play a role as anti-tumor cytokines.

One study tested the possibility of whether IL-32 could be developed as an anti-tumor drug. To evaluate the anti-tumor effect of IL-32 gene therapy, CMS4 sarcoma-bearing Balb/c mice were established and they were received intratumoral injections of syngeneic DCs engineered to overexpress human IL-32β. IL-32β-overexpressing DCs more potently activated the type 1 T cell responses *in vitro* and *in vivo*, leading to CTL-dependent suppression of tumor growth, which was independent of $CD4^+$ T and NK cells (72). Contrary to the xenograft model using IL-32β-overexpressing SW610 and PC3 cells, the activity of NK cells was not affected. Thus, IL-32 could have a selectivity for CTL or NK cells to exert anti-tumor activity in a context-dependent manner. Collectively, IL-32 directly inhibits tumor cell growth via inhibition of NF-κB and STAT3 in tumor cells, recruits anti-tumor immune cells, including NK cells and CTLs, and also directly regulates the cytolytic activity of NK cells via increases in activating ligand and death-inducing ligands.

3.3 IL-32 stimulates differentiation of immune suppressor cells

Ohmatsu et al. showed that IL-32 was highly expressed in MF lesions and accelerated the induction of $CD11c^+$ mature DCs (mDCs) and $CD163^+CD68^+$ macrophages (MΦ) from monocytes. The differentiated cells expressed immunosuppressive indoleamine 2, 3-dioxygenase (IDO) and IL-32. On the other hand, increased expression of IL-10 across MF lesions was highly correlated with IL-32 and IDO, but not with Foxp3 expression. Thus, in addition to a stimulatory effect on tumor proliferation, upregulated IL-32 in MF lesions might foster an immuno-tolerogenic environment by induction of immunosuppressive mDCs or MΦ by increasing immunosuppressors, such as IL-10 and IDO (73). Effects of IL-32 on the recruitment of NK cells and CTLs, and differentiation of immune suppressor cells in the tumor microenvironment are described in Figure 3B.

4. Effect of IL-32 on stem cells

It is well known that octamer-binding transcription factor 4 (OCT4) expression, one of stemness factors, is increased in cancer stem cell (CSC)-like cancer cells and enhances CSC-like characteristics, including sphere formation, cell colony formation, cell migration, invasiveness, and drug resistance. Patient-derived CRCs enforced to express OCT4 resulted in high expression of IL-8 and IL-32. Neutralization of either IL-8 or IL-32 in those CRCs partially inhibited the tumorigenic effects exerted by OCT4 overexpression. Altogether, these data indicate that IL-32 affects the regulation of CSC-like properties with IL-18, through both autocrine and paracrine manners (74). To furtherly expand on this concept, more patient-derived CRCs should be established because the study developed CRCs from only two patients. Future studies should be undertaken to determine whether OCT4 is a direct transcription factor inducing IL-32, and which IL-32 isoform is associated with induction by OCT4.

In an effort to identify a new growth factor which stimulates proliferation of hematopoietic progenitor cells (HPCs), Moldenhauer et al. compared the gene profile of human endothelial cells (ECs) before and after IL-1β treatment. Notably, IL-32 significantly induced the proliferation of HPCs. Furthermore, IL-32 attenuated chemotherapy-related bone marrow cytotoxicity by increasing the number of HPCs in mice (75). Thus, if we consider a report showing that IL-32 can play as an autocrine factor for cancer stemness (74), IL-32 seems to regulate the stemness of hematopoietic stem cells, too.

CONCLUTION

In various tumor cells, IL-32 is highly increased and mainly located in the cytoplasm. Presently, IL-32β is considered the primary isoform in cancers. By thorough review of published studies on the biological roles of IL-32 in various cancers, we found several points that should be considered for further studies. First, whether the functional roles of IL-32 in cancer are dependent on cytosolic or extracellular IL-32 should be investigated. Second, it is important to elucidate the critical signal that allows IL-32 to act as a pro-tumor or anti-tumor cytokine. Third, it is important to determine whether IL-32 originates from stromal or tumor or immune cells to comprehensively understand the role of IL-32 in the formation of tumor microenvironments.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

FIGURES LEGENDS

Figure 1. Biological functions of IL-32 in inflammatory tumors. Different colors indicate each cancer type and cancer type specific signaling pathway. AOM: azoxymethane, ROS: reactive oxygen species, JNK: c-Jun N-terminal kinases, NF-κB: nuclear factor kappa B, TIMP: tissue inhibitors of metalloproteinase, MMP: matrix metalloproteinase, *H. pylori*: *Helicobacter pylori*, cagPAI: cytotoxin-associated gene pathogenicity island, HBV: hepatitis B virus, HCV: hepatitis C virus, VEGF: vascular endothelial growth factor, CXCL: C-X-C motif chemokine, CCL: C-C motif chemokine ligands, TNF-α: tumor necrosis factor α, PI3K: phosphoinositide 3-kinase, AP-1: activated protein 1.

Figure 2. Biological functions of IL-32 in non-inflammatory tumors. Different colors indicate each cancer type and cancer type specific signaling pathway. *H. pylori: Helicobacter pylori*, COX2: cyclooxygenase 2, ROS: reactive oxygen species, HPV E7: human papillomavirus E7 oncoprotein, PARP: poly ADP-ribose polymerase, VEGF: vascular endothelial growth factor, pSTAT3: phosphorylated signal transducer and activator of transcription 3, PKCδ: protein kinase C δ.

Figure 3. IL-32 affects both tumor growth and death by modulating immune cells. A. IL-32 stimulates tumor and NK cells to facilitate tumor cell killing. Expression levels of NK cell target proteins, including FAS and ULBP are upregulated in IL-32α-expressing CML (upper). IL-32-expressing NK cells secrete the ligands for DR3 and TNFR2, DR3 and TNF, respectively (bottom). B. IL-32-expressing tumor cells recruit

anti-tumoral NK cells and CTLs, while IL-32 also stimulates the differentiation of immunosuppressive CD11c+ mDC and CD163+CD68+ MΦ. FASL: Fas ligand, ULBP2: UL16-binding protein 2, NKG2D: natural killer group 2D, CML: chronic myeloid leukemia, NK cell: natural killer cell, TNFR2: tumor necrosis factor receptor 2, DR3: death receptor 3, CTL: cytotoxic T lymphocytes, MΦ: macrophages, mDC: mature dendritic cells.

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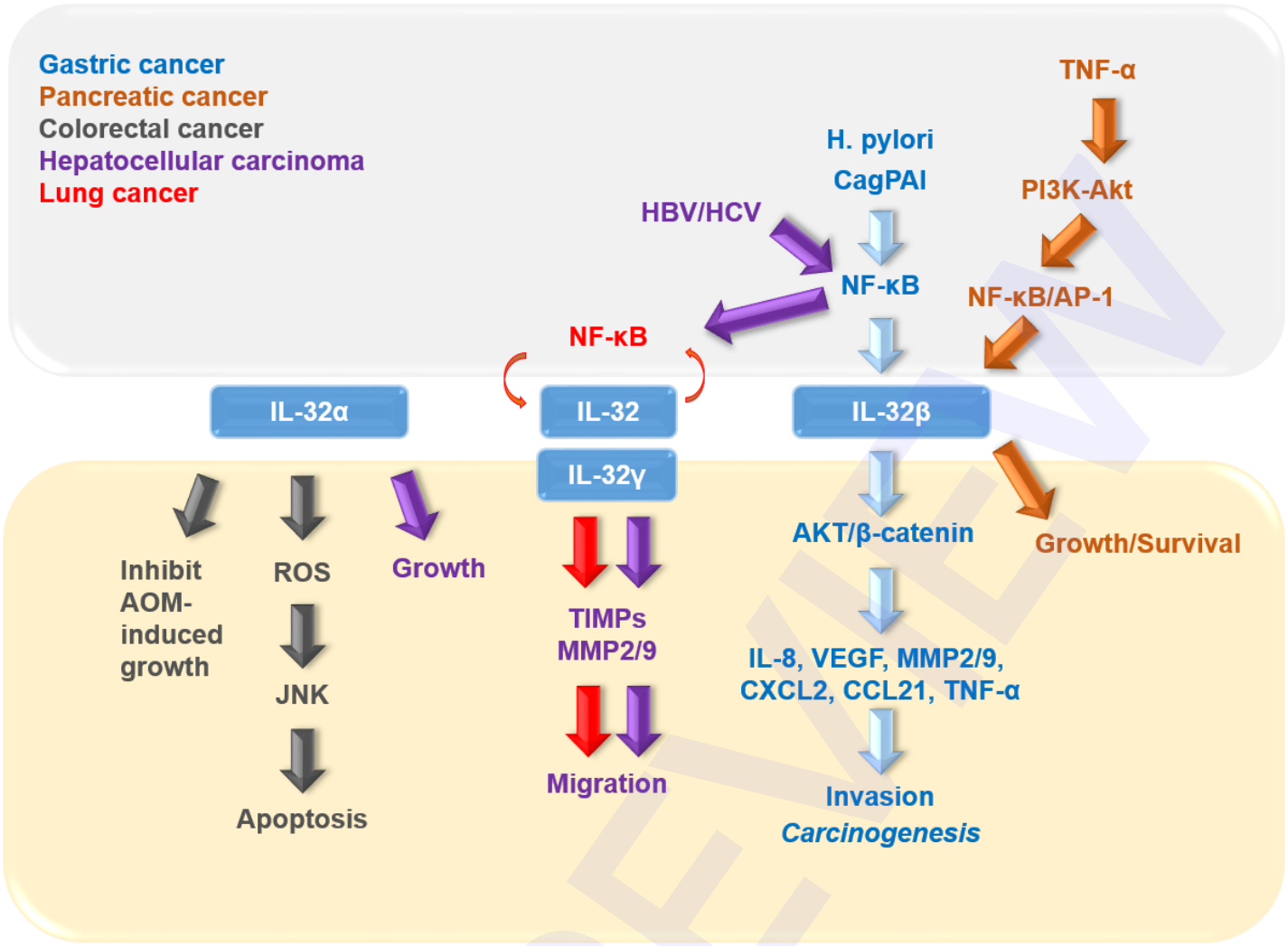
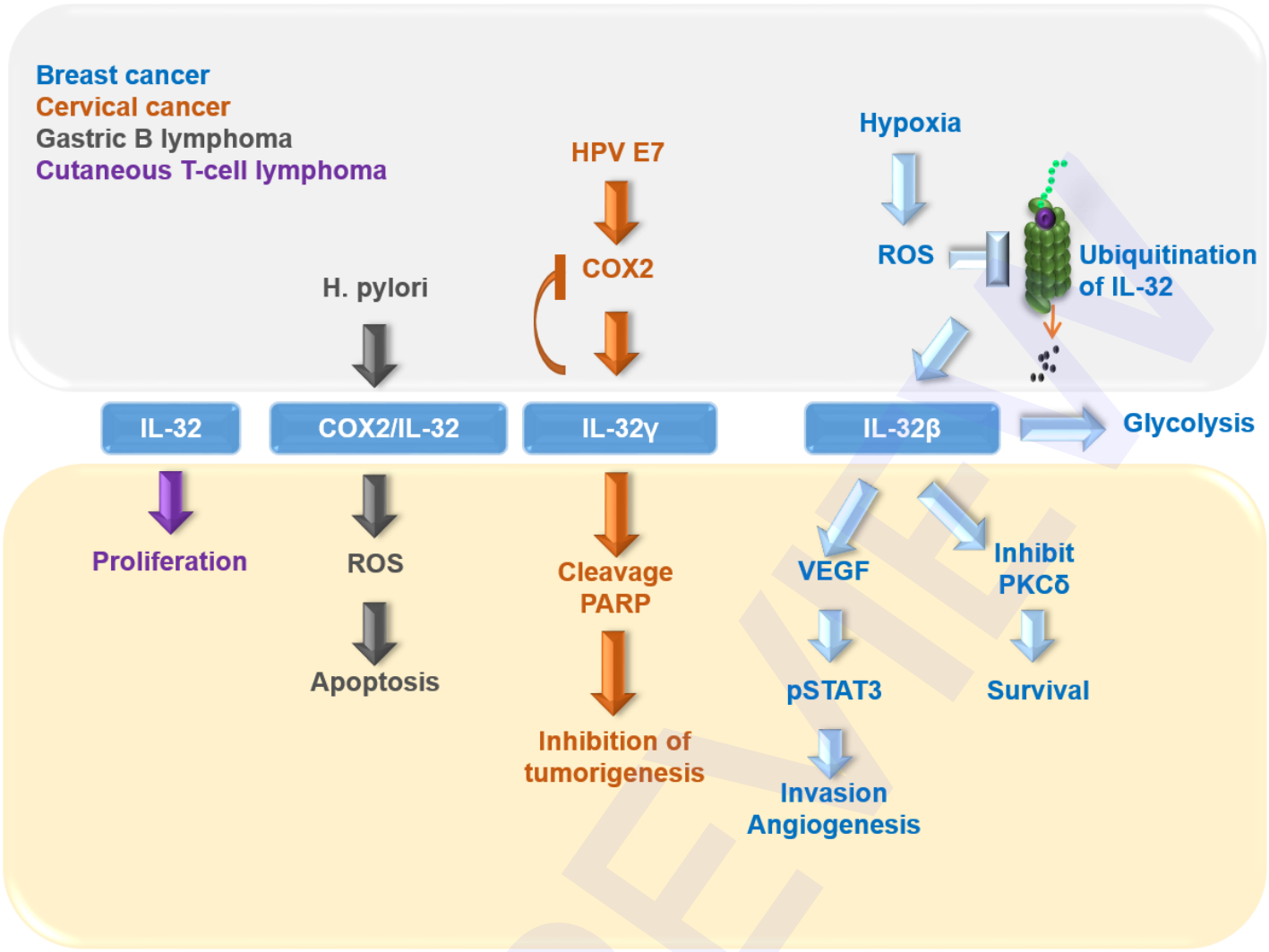


Fig. 1.



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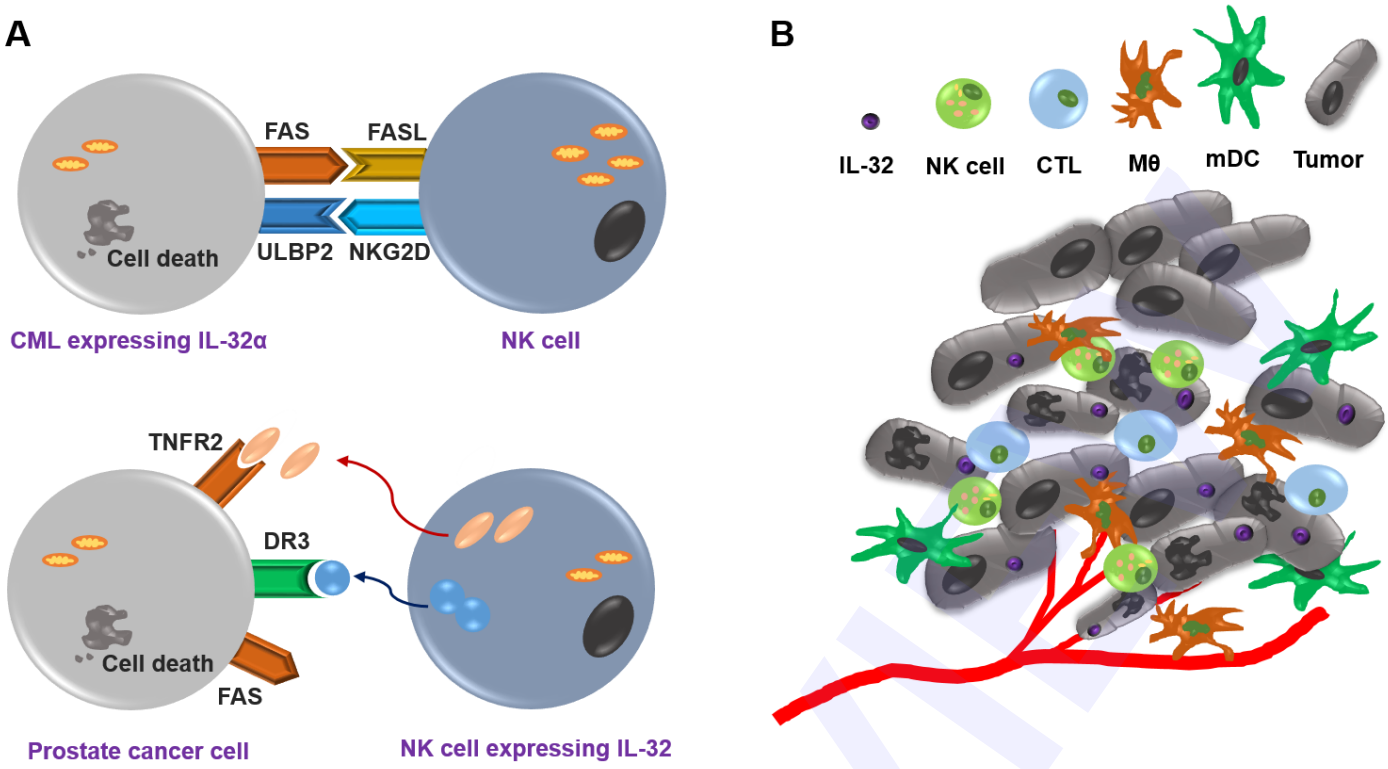

Fig. 3.