REVIEWS

The Role of IL-35 in Regulating Tumor Immunity

Junfeng Zhang1, Yunsheng Zhang1, Chunlei Li4, Xiaomei Zhang6, Qingpeng Wang3, Huabao Xiong1,5,* and Hongxin Deng2,*

1 Institute of Immunology and Molecular Medicine, Jining Medical University, Jining, Shandong, 272067, China.
2 Cancer Center, West China Hospital, Sichuan University, Chengdu, Sichuan, China.
3 Institute of Biopharmaceutical Research, Liaocheng University, Liaocheng 252059, P.R. China.
4 School of Pharmacy, Linyi University, Linyi, Shandong, 276000, China.
5 Department of Medicine, Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.
6 Laboratory Animal Center, Sichuan University, Chengdu, Sichuan, China.

Abstract: Interleukin 35 (IL-35) is the newest identified heterodimeric cytokine in the IL-12 cytokine family. IL-35 is a heterodimer comprised of IL-12 subunit alpha (p35) and IL-27 subunit Epstein-Barr virus-induced gene 3 (EBI3). Since discovery, IL-35 showed different immunosuppressive functions from other members of the IL-12 family. It is shown that IL-35 inhibits immune responses by suppressing the differentiation of Th17 cells and macrophages and inhibiting the proliferation and function of effector T cells. Although studies showed that IL-35 has been secreted by regulatory T cells, CD8+ regulatory T cells and regulatory B cells, other studies have also shown that IL-35 may be more widely expressed in cancer cell lines, such as lung adenocarcinoma, hepatocellular carcinoma, cervical carcinoma, breast cancer, and gastric cancer. Recent studies have also shown that IL-35 secreted by tumor cells promotes both tumor growth and metastasis by enhancing the accumulation of angiogenesis and myeloid-derived suppressor cells, and decreasing the infiltration of CD8+ T cells into tumor microenvironment. In this review, the structure and biological functions of IL-35 were reviewed in detail, and the roles of IL-35 in different types of cancers including colorectal cancer, pancreatic ductal adenocarcinoma, acute myeloid leukemia, hepatocellular carcinoma, breast cancer and so on were discussed.

Keywords: Interleukin 35; Cancer; IL-35 signaling; Microenvironment

Corresponding Author(s): Correspondence to: Hongxin Deng, Cancer Center, West China Hospital, Sichuan University, Ke-yuan Road 4, No. 1, Gao-peng Street, Chengdu, Sichuan, 610041, the People’s Republic of China; Tel: +86 028 85164063; E-mail: deng-hongx@scu.edu.cn or Huabao Xiong, Department of Medicine, Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. Phone: 212 659 9585; Fax: 212 849 2525; Email: huabao.xiong@mssm.edu.

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1. Introduction

The tumor microenvironment has many characteristics, including supporting angiogenesis and immune escape mechanisms[1]. Many of these mechanisms that inhibit the immune function of tumors are important for the body’s immune system to maintain stability[2]. These regulatory mechanisms play an important role in the process of autoimmune regulation after anti-infection and injury[3,4]. Treatment of tumors by immunological methods has manifested superior successful outcomes compared with traditional chemotherapy modalities[5]. The basis of this treatment relies on the notion that immune system often recognized these cancer cells as abnormal extraterrestrials, and enhancing the immune response to cancer cells can translate into cancer treatment response[5,6].

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Activated T cells are mainly divided into CD4+ T cells and CD8+ T cells. Among them, CD4+ T cells are mainly divided into Th1 (CD4+IFN-γ+), Th2 (CD4+IL-4+), Th9 (CD4+IL-9+), Th17 (CD4+IL-17+), Tr1 (CD4+IL-10+) and Treg (CD4+CD25+Foxp3+) cells according to the expression of cytokines[28]. Antigen presenting cells (APCs) are divided into professional antigen presenting cells and nonprofessional antigen presenting cells. The professional APCs are the main APCs, which contain dendritic cells (DCs), B lymphocytes and monocytes/macrophages[9,10]. IL-35, together with IL-23 and IL-27 belong to the IL-12 family, which characterized by heterodimeric members is formed by alpha chains (p35) and beta chains (EBI3)[11,12,29]. Unlike other IL-12 family members, which are known to be mainly secreted by activated antigen-presenting cell (APCs)[13-15], IL-35 is primarily expressed in the non-stimulated regulatory T cells (Tregs) in mice, but is not detected in the non-irritating human Tregs[16]. Recently, IL-35 was also proved to be secreted by a wide range of tissues and different cell types, including dendritic cells (DCs)[17], regulatory B-cells[18,19], sometimes, in endothelial cells, smooth muscle cells, and monocytes[20].

Recently, researches have demonstrated that IL-35 widely shows its inhibitory effect on T cells proliferation both in vivo and in vitro[11]. What's more, the transformation of traditional T cells into inducible Tregs (iTregs) induced by IL-35, inhibiting Th17 cell differentiation and development[21,22]. IL-35 also suppresses the development of autoimmune diseases and inflammatory diseases[23-25]. Recent results have also found the effect of IL-35 in inducing IL-10-producing B cells and promoting the transformation of Breg cells into IL-35+ Breg cells, which play an important role in cancers[26].

2. The Discovery and Composition of IL-35

In 1997, Devergne et al. found that EBI3 and IL-12p35 could polymerize into new hematopoietic factors in vivo. However, no functional researches have been carried out in the next 10 years. It is also the first article to discover that EBI3 and IL12p35 can form a new heterodimeric[26]. It was until 2007 that the heterodimeric was officially named IL-35 at the thirteenth immunology conference. In the same year, Collison et al. demonstrated that the immunosuppressive cytokine IL-35 is the latest member in IL-12 family and plays an important regulatory role in Tregs for the first time[11]. The IL-12 family consists of four members: IL-12, IL-23, IL-27 and IL-35. All of these cytokines are heterodimeric, in which p40 and p35 constitute IL-12, p40 and p19 to form IL-23, IL-27 is composed of EBI3 and p28, while IL-35 contains p35 and EBI3 two subunits (Fig.1). EBI3 plays an important role in the immune response. It can downregulate the expression of Th17 cell transcription factor RorγT and have the effect of inhibiting inflammation in inflammatory response[27]. p35 subunit can cause herpes simplex keratitis in mice, and it has an important effect in regulating inflammation[28]. Each of the two subunits of IL-35 has the function of regulating immune response. When they form heterodimeric, they play immunoregulatory roles in the form of IL-35.

In the past, it was found that IL-12 family members except IL-35 were mainly secreted by APCs, including macrophages, DCs, and monocytes[12,29]. The initial study found that only the Tregs of the Foxp3+ cells could secrete active IL-35, and the effect T cells (Teffs) did not secrete it[19]. In human, IL-35 was not detected in the unstimulated Treg cells, but the activated ones could be detected the secretion of the IL-35[20,30]. However, researchers discovered that rhinovirus activated DCs which could secrete and release IL-35 into human peripheral blood. In 2014, other researchers further found that B cells also secrete IL-35, and these B cell has immunosuppressive function in both autoimmune and bacterial infections[19]. These results indicate that different stimulus and immune microenvironment may affect the production of IL-35 cytokines.

3. IL-35 Receptors and Signaling Pathway

As mentioned above, IL-12, IL-23, IL-27, and IL-35 all contain two subunits. The IL-12 family of receptors are also contain several subunits: IL-12Rβ1, IL-12Rβ2, WSX, gp130, and IL-23R (Fig.1)[12]. Unlike other IL-12 family members, the receptors of IL-35 contain gp130-gp130, IL-12R-β2-IL-12R-β2, IL-12R-β2-gp130, and IL-12R-β2-IL-27R (WSX-1) (Fig.1)[27]. In general, most gp130 is secreted by immune cells, while IL-12Rβ2 subunit is mainly produced in NK, active T cells, and a lesser extent DCs.
and B cells\(^{[33,34]}\). Following IL-35 binding to its receptors, its signal transduction through the unique heterodimer formed by STAT1 and STAT4, which lead to the target genes expression, including EB13 and p35. These target genes lead to the feedback loop promoting the expression of IL-35\(^{[22]}\). The IL-35 signaling pathway is consisted of STAT1, STAT3, STAT4, JAK1, and JAK2 molecules (Fig.1)\(^{[13,56]}\). The IL-35 signals contain three receptor subunits in T cells, including gp130-gp130, IL-12R-β2-IL-12R-β2, and IL-12R-β2-gp130 that activate the STAT1 and STAT4 molecules\(^{[37,38]}\). IL-35-producing regulatory B cells have two receptor subunits, including IL-27R-α and IL-12R-β2, which activate STAT1 and STAT3 signaling molecules\(^{[19,39]}\). These results showed that the binding of IL-35 to different receptors subunits depends on different type of cells.

4. Main Biological Effects of IL-35

In the past ten years, there has been no interruption in the study of IL-35. Researchers found that IL-35 has three major biological effects in a variety of disease models: (1) Inhibition of T cells proliferation. It has been found that exogenous IL-35 can inhibit Th1 and Th17 type cells in mice and promote CD39 antigen expression in CD4+ T cells. At the same time, the authors found that the CD4+CD39+CD25- T cells isolated from the mice in the IL-35 treatment group could express high IL-10, but the CD4+CD39+CD25+ T cells could not be induced\(^{[23,40]}\). (2) Naive T cells transformation into IL-35 induced regulatory T cells (iTr35 cells). IL-35 plasmid can effectively treat allergic airway inflammation induced by memory/effector Th2 cells from specific allergen mite, and this treatment is accomplished mainly by regulating inflammatory factors expression (including IL-4, IL-5 and IL-13,) and chemokine (including CCL2, CXCL1, and CXCL5)\(^{[16,23,41]}\). (3) Down-regulate the development and differentiation of Th17 cells. IL-35 has been shown to control the Tregs phenotype and suppress the Th17 cells differentiation, and thereby inhibit immune challenge against β-cells in patients with type 1 diabetes\(^{[32,43]}\). In these studies, IL-35 plays an important role in inflammatory diseases and autoimmune diseases. In addition, our group found that IL-35 could alleviate pathological characteristics of severe psoriatic lesions in K14-VEGF-A-Tg mice by reducing the ratio of the total number of macrophages and M1/M2 macrophages that has not been reported previously\(^{[24,45]}\). However, in recent years, it has been found that the immunomodulatory effect of IL-35 in tumor microenvironment is also very important. Next, we will introduce the immune function of IL-35 in cancer in detail.

5. IL-35 and Cancers

Tregs is one of the main obstacles to effective immunotherapy of tumor and depletion Tregs can effectively inhibit tumor proliferation\(^{[46]}\). Inflammation is one of the ten characteristics of cancer, researchers believe that IL-35 might influence the tumor inflammation\(^{[47]}\). Thus, whether IL-35 as a cytokine mainly secreted by Tregs can regulate tumor microenvironment has also been reported in recent years. We searched PubMed for papers published before 2018 with the keywords “IL-35 and cancer”. Relevant papers were included after manual selection based on the abstracts and discussion within the research groups. The main point is that IL-35 in the tumor microenvironment can increase tumor immune escape, limit anti-tumor immunity, and decreased survival of the immunocompetent tumor-bearing host\(^{[46]}\).

5.1 IL-35 and Colorectal Cancer

Development of colorectal cancer (CRC) is closely related to inflammation. EB13 is a subunit of the IL-12 cytokine family that not only forms the IL-27 heterodimer with IL-27p28 subunit, but also forms IL-35 with the IL-12p35. However, IL-27 promotes while IL-35 mainly inhibits T cells responses\(^{[11,49]}\). Recent study has found that blocking EB13 can inhibit the proliferation of colorectal cancer cells and the growth of tumor, which promotes the response of cytotoxic T lymphocyte (CTL) by inducing Granzyme B, the production of IFN-γ and the expression of p-STAT3\(^{[50]}\). Another study has shown that IL-35 is highly expressed in both colorectal cancer cells and peripheral blood of patients, whereas these high levels of IL-35 are closely related to the degree of malignancy and clinical stage of CRC\(^{[51]}\). Similarly, Yanhui Ma's results showed that high expression of IL-35 in serum and tumor microenvironment in CRC was closely related to tumor metastasis. Meanwhile, the high level of IL-35 in colorectal cancer promoted IL-35 production through STAT1 and STAT3, which inhibited T cells proliferation\(^{[32]}\). In the progression of CRC patients, IL-35-producing B cells also increased significantly, which is critically correlated with the proportion of CD4+CD25+/highCD127low/-- Treg cells\(^{[53]}\). These results indicated that IL-35 might participate in the immune suppression of CRC. However, Jianli Zhang’s team showed the opposite results. Their study found that compared with paired normal tissues, the expression of IL-35 was significantly reduced in CRC tissues, and this phenomenon was closely related to the metastasis and low survival rate of CRC\(^{[54]}\). These results suggest that the regulatory role of IL-35 is very important in colorectal cancer.
5.2 IL-35 and Pancreatic Ductal Adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC) is one of the most common human cancers. Invasive human tumors are very refractory to standard treatments and are almost deadly. Recent studies have shown that the subunits of IL-35 are highly expressed simultaneously in many human cancer cell lines compared with normal cells, including lung adenocarcinoma, colon cancer, breast cancer, hepatocellular carcinoma, cervical cancer, and pancreatic cancer. Study showed that IL-35 promoted the growth of pancreatic cancer cells lines through inhibiting their apoptosis. IL-35 induced the proliferation of these cancer cell lines by increasing the expression of cdk2, cdk4, cyclin B, and cyclin D. Researchers found that the expression of IL-35 in the plasma of PDAC was higher than that of normal controls. Chongbiao Huang et al. also demonstrated that IL-35 is highly expressed in tumor tissues of patients with PDAC, and overexpression of IL-35 is associated with poor prognosis in PDAC patients. What's more, IL-35 could mediate endothelial cells adhesion and extravasation through a GP130-STAT1 signaling pathway. These results showed that regulating IL-35 expression in PDAC patients might provide a new target for the pancreatic ductal adenocarcinoma treatment.

5.3 IL-35 and Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is one of the most common malignant tumors in adults. It is mainly caused by abnormal development of bone marrow or bone marrow precursor cells in blood or bone marrow. The mechanism of Tregs mediated tumor immune escape is one of the main causes of AML. As a new type of suppressor cytokine, IL-35 is specifically expressed by Tregs, which in turn regulates the function of Tregs. However, IL-35 expression in human Tregs is still controversial, and its role in AML still to be explained until 2012. In 2012, for the first, Chunyan Ji et al. demonstrated that elevated levels of IL-35 in plasma in newly diagnosed AML patients suggested that the cytokines participate in the pathophysiology of the disease. Then, Zhimin Zhai's team used many methods to detect the expression of IL-35 in bone marrow of adult AML patients, and found that IL-35 was also highly expressed in bone marrow. Treatment of AML with IL-2-diphtheria fusion protein showed that the expression of IL-35 was significantly reduced. The above studies only detected the level of IL-35 in AML patients, and did not explore the mechanism. Another study demonstrated that Tregs isolated from AML patients could secrete IL-35 under stimulation. Meanwhile, IL-35 promotes the immune escape of AML cells by enhancing the function of Tregs and inhibiting the effect of Teffs. IL-35 can also promote the proliferation of AML cells directly and reduces AML cells apoptosis. These studies showed that IL-35 could promote the progression of AML cells and have an important regulatory role in the development of AML.

5.4 IL-35 and Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is a malignant tumor that occurs in the liver and has a high mortality rate. Its incidence is very high, especially in China. It is mainly divided into two major categories: Primary hepatoma originates in the liver epithelial or mesenchymal tissue and shows high incidence and malignancy. Secondary hepatoma is called sarcoma and is relatively rare compared with primary hepatoma. Recent study demonstrated that IL-35 was constitutively expressed in HCC patients as an immunosuppressive cytokine. In addition, they found that higher expression of IL-35 was detected in advanced Barcelona clinic liver cancer stage patients and significantly higher risk of cancer recurrence. Conversely, Yaozhong Ding's group had revealed that low IL-35 expression in HCC tissues might play an important role in the development of HCC. Meanwhile, over-expression of IL-35 in hepatoma cells (HepG2) could enhance apoptotic sensitivity and induce cell cycle arrest of HCC through the regulation of genes related to the cell cycle and apoptosis, including a rise of Fas and down-regulation the expression of cyclinD1, survivin, and Bcl-2. In the recent study, they also proved that overexpression of IL-35 significantly decreased the HepG2 cells migration and invasion by reducing the produce of MMP-2 and MMP-9. The Notch signaling pathway is a conserved structure. Researchers have found that the Notch signaling pathway is not only involved in the development, but also has an important effect in the occurrence and development of tumors for individual organisms. Activated Notch signaling pathway in hepatocellular carcinoma cell lines were found. Therefore, understanding the mechanism of Notch signaling pathway during invasive migration can help people solve a lot of problems in the treatment of liver cancer. Q. SH. Wang et al. found that the combination of IL-17 monoclonal antibody Secukinumab and IL-35 could reduce the invasion and metastasis of hepatoma cells by blocking the Notch signaling pathway.

5.5 IL-35 and Breast Cancer

In recent years, Breast Cancer (BC) is one of the most rapidly increasing morbidity and mortality, and the most serious malignancies in women. The annual incidence is about 1.7 million and the mortality rate is 500,000.
Prognostic parameters such as serum tumor biomarkers, and lymph node staging were used for the estimate of BC prognosis. However, the survival rate of BC after treatment is still not satisfactory. The 5-year survival rate for early diagnosis of BC can be as high as 90%, but the ratio is significantly reduced to 20% once the tumor metastasizes.[77] Recently, Chen et al. reported all known subunits of the IL-12 family, including EBI3, IL-12p35 and IL-12p40 subunits were highly expression in breast cancer tissues, and the systemic levels of IL-35 and IL-27 were also higher compared with healthy controls. Meanwhile, a positive correlation was reported among the IL-35/IL-23 ratio in the blood of BC patients.[78]. Almost at the same time, Zhao et al. indicated that the increased expression of IL-35 in BC tissues may play an important role in the prognosis and development of BC. In addition, IL-35+ Treg cells can induce the formation of Bregs in the BC microenvironment, and the IL-35 secreted by these Breg can also regulate Treg or CD8+ Treg cells.[79]. These above data demonstrated that increased IL-35 in BC tissues could inhibit the anti-tumor immune response and leads to the occurrence, development and progression of BC. It is suggested that IL-35 has an important immunosuppressive effect on breast cancer as an immunosuppressive cytokine in the BC microenvironment.

5.6 IL-35 and Other Cancers

In addition to several tumors mentioned above, the expression and biological functions of IL-35 in several other tumors were also studied. Researchers believed that Foxp3+ Treg and other Tregs (such as Breg and CD8+ Treg cells) are the main source of IL-35 in the tumor microenvironment. This IL-35 can effectively inhibit the proliferation and differentiation of immune cells, including Th1, Th2 and Th17 cells.[21]. It is also found that the subunit EBI3 of IL-27/IL-35 plays an important role in the occurrence, development and poor prognosis of lung cancer tumors.[80]. In the collagen-induced arthritis (CIA) model of rheumatoid arthritis (RA), exogenous IL-35 is able to inhibit the expression of mediators of angiogenesis through STAT1. This is a provide mechanism for anti-angiogenic effects found in experimental models of RA. These data suggest that IL-35 and its signaling pathways may be key factors in the treatment of vascular related diseases such as RA.[80]. A similar study has found that IL-35 could inhibit angiogenesis and inflammation in RA by down-regulating the expression of VEGF-induced Ang2 and disturbing Ang2/Tie2 signaling.[81]. Does IL-35 affect tumor angiogenesis in tumors? Conversely, IL-35 produced by tumor cells can promote the tumor growth by promoting the recruitment of CD11b+Gr1+ myeloid-derived suppressor cells and increase angiogenesis in the tumor microenvironment.[60]. The levels of IL-35 in plasma were significantly increased in prostate cancer patients, which could be used as a biomarker for tumor biopsy in early stage of prostate cancer patients.[82]. However, Olsen et al. reported that blocking IL-35 could inhibit the antigen-specific Treg cells of human prostate tumor.[83]. The expression of circulating IL-35 was significantly higher in non-small cell lung cancers, which showed that IL-35 may be a predictor of non-small cell lung cancers.[84].

6. Conclusion

In recent years, many studies have shown that IL-35 has an important effect in the occurrence, development and prognosis of various tumors. It has been mainly confirmed that IL-35 can not only promote tumor proliferation and progression by a variety of mechanisms such as promoting angiogenesis, but also inhibit tumor cell apoptosis by regulating apoptosis genes (Fig. 2). Moreover, IL-35 can also promote tumor growth through a variety of mechanisms, such as inhibiting Teffs and promoting proliferation of Tregs and accumulation of the CD11b+Gr-1+ myeloid cells (Fig. 2). A large number of studies have showed that IL-35 is not only secreted by Treg cells, as well as new cell sources, such as Breg cells, CD8+ Treg cells, and dendritic cells. The immune-modulatory function of IL-35 has been further explored. These different sources of IL-35 appear to be used as a biomarker for diagnosis of many cancers. An in-depth study on the expression and regulation mechanism of IL-35 will contribute to the development of a new kind of immunotherapy for cancers.

References


