

Interleukin-12 Implication in Tumor Initiating Capacity of Colorectal Cancer Stem Cells

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Introduction

Colorectal cancer (CRC) is the fourth most common malignancy worldwide, with 1.23 million patients diagnosed each year and a huge proportion of them characteristic early metastasis [1]. Usually, colorectal cancer mortality occurs in patients whose cancer cells metastasize to distant sites, such as the liver, lungs, brain, and bones. In detail, individual cells must reach the sites of metastasis and proliferate to form secondary tumors. However, only a small proportion of cancer cells in primary colorectal tumors have self-renewal and tumor initiating capacity, which is necessary to form metastasis or recurrent tumors, and are designated as tumor initiating cells or cancer stem cells (CSCs).

CSCs play a critical role in metastatic process, which is the major cause of death for colorectal cancer patients. Not only because CSCs are capable of forming clinically relevant metastases at secondary sites, but also, the CSCs have increased resistance to chemotherapy and radiation therapy [2,3]. Therefore, newly effective therapeutic strategies especially targeting CSCs are urgently required. As a strategy for cancer therapy, immunotherapy has begun to revolutionize cancer treatment, by introducing therapies that targeting not the tumor itself, but the host immune system, therapies that possess unique adverse event profiles, and therapies that might monitor cancer stem cells in many different types of cancer [4]. Despite recent advances in the development of cancer immunotherapies and vaccines, immune suppression and tumor tolerance remain formidable obstacles to many potentially effective immunotherapies.

Cytokine disbalance which exists in most human cancers, is the main cause of immune system function disorder that might lead to tumor occurrence and development [5]. Interleukin (IL)-4, IL-6 and IL-8 levels elevated in most patients suffering from breast, prostate and colorectal cancers. In contrast, the level of IL-12 was too low to be detected in the sera of patients with gastric and colorectal cancer [6]. IL-12 effectively enhances anti-tumor immune response but the function of IL-12 in regulating CSCs survival and invasiveness is not clear. In our previous published article entitled "Interleukin-12 inhibits the survival of human colon cancer stem cells in vitro and their tumor initiating capacity in mice" Xiaoling Yin et al. report the first evidence of the IL-12 implication in CSCs phenotype [7]. Interleukin 12 (IL-12) plays an important role in creating an interconnection between the innate and adaptive immunity, such as induces the Th1-type immune response [8]. It is naturally produced by dendritic cells [9], macrophages, neutrophils, and human B-lymphoblastoid cells in response to antigenic stimulation. IL-12 has been observed in many different types of cancer, such as cutaneous T-cell lymphoma, renal cell carcinoma, ovarian cancer and melanoma. IL-12 binds to the IL-12 receptor to active its potential signal transduction. IL-12 receptor is a

heterodimeric receptor formed by IL-12R- β 1, and IL-12R- β 2 which is considered to play a key role in IL-12 functions [10]. As an antitumor cytokine, IL-12 is involved in many aspects of antitumor responses, such as stimulation of growth and cytotoxicity of activated NK cells, CD8+ and CD4+ T cells [11], shifting differentiation of CD4+.

Th0 cells toward the Th1 phenotype [12]; increasing production of IFN- γ , which is the most potent mediator of IL-12 actions, from NK and T cells [13]; enhancement of antibody-dependent cellular cytotoxicity (ADCC) against tumor cells [14,15]; and the induction of IgG and suppression of IgE production from B cells [16]. Apart from IL-12, IL-4 is usually secreted by activated T-cells and has shown antitumor activity in vitro against lymphoma, multiple myelomas, chronic myelomonocytic leukemia and some solid tumors [17]. Previous studies have showed that the protection from apoptosis is mediated by stem-like cell autocrine production of IL-4 through up-regulation of an anti-apoptotic mediator surviving [18]. IL-4 has been involved in the regulation of survivin expression as well as its localization through activating critical transcription factor proteins such as STAT-6 [19]. Marshall et al. [20] also shown that IL-12 inhibits the production of IL-4 in human CD4+ T lymphocytes, but its implication in IL-4 secretion from cancer stem cells has not been tested yet.

Yin et al. [7] investigated the expression of IL-12 in human sera from colon cancer patients by ELISA, and found that IL-12 expression was reduced in human colon cancer. In addition, lower expression of IL-12 was correlated with colon cancer progression. They next transfected lentivirus-IL-12 into the colon CSCs and found that IL-12 expression decreases tumorsphere formation of colon CSCs in vitro. In vivo experiments showed that IL-12 inhibits tumor growth initiated by CSCs in NOD/SCID mice. Additionally, they revealed that IL-12 suppresses the IL-4/STAT6 signalling pathway in colon CSCs. In this study, Yin et al. report the capacity of IL-12 to regulate the self-renewal and differentiation of human colon CSCs in vitro, as well as its inhibition of tumors initiated by CSCs in mice. But why did IL-12 capture their function in CSCs? Previous study in mesenchymal stem cells (MSCs) revealed that lentivirus-mediated IL-12 genetically modified MSCs and inhibited malignant ascites by stimulating the immune responses of the mice [21]. Furthermore, the rates of apoptosis and proliferation were affected by IL-12 transfection. Yin et al. [7] demonstrated that a higher number of apoptotic cells were detected in lentivirus-mediated IL-12 transfected CSCs. These results suggest that the loss of IL-12 expression in colorectal cancer probably contributes to reduced immune responses as well as increased cell proliferation and migration during tumor development.

Since IL-12 is one of the important regulators in controlling immune responses, IL-12-based approaches for cancer therapy have been emerged and developed during the past two decades. Emerging

evidences have demonstrated that IL-12 is one of the most potent cytokines in mediating antitumor activity in a variety of preclinical models [22]. However, the robust antitumor effect exerted by IL-12, has not yet been successfully translated into the clinics. The majority of clinical trials involving systemic administration of IL-12 failed to show sustained antitumor responses and was associated to toxic side effects [22]. On the contrary, local application had a promising safety profile at better antitumor efficacy, especially in solid tumors. In addition, combination of IL-12 with other antitumor drugs, such as cyclophosphamide [CPA] or anti-CD25 mAb, showed better antitumor efficiency especially in colorectal carcinomas [23].

As results from the scientific research always lead to new questions, the next step is to elucidate the mechanism in which IL-12 activates on CSCs. Previous studies have shown that the protection from apoptosis is due to stem-like cell autocrine production of cytokine IL-4. IL-4 is known to be a pleiotropic lymphokine which plays a central role in the regulation of immune system. IL-4 activates its potential signalling pathways through tyrosine phosphorylation of STAT6, a signal transducer and activator of transcription [24]. Yin et al. revealed that CSCs expressed increasing levels of IL-4 and p-STAT6, suggesting the possible existence of autocrine IL-4 and p-STAT6 signaling loop in CSCs. Previous studies have shown that IL-4 inhibits the production of IL-12 by STAT6-dependent and independent mechanisms [25]. In our study, IL-12 expression led to a 60% reduction in the protein level of IL-4 in colon CSCs, in association with apoptosis but reduced proliferation. Although this may explain the capacity of IL-12 to directly inhibit the stemness of CSCs, further studies will be needed to demonstrate this mechanism more convincingly. We believe that upcoming discoveries in this area are promising. Undoubtedly, future research work will aid us in better understanding the complex mechanisms of IL-12 and IL-4/STAT6 signaling and colorectal cancer stemness phenotype.

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