Review Article

Interleukin-4 and Breast Cancer

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IL-4 is a pleiotropic cytokine produced by T lymphocytes which acts on various cells of such as T and B lymphocytes, monocytes, fibroblast, endothelial cells, macrophages and some others. IL-4 was originary described as a B cell growth factor, and now known to provide potent anti-tumor activity against various tumors, including breast cancer. IL-4 can induce apoptosis in cultured breast cancer cells. In addtion, it has been clarified that IL-4 plays an important role in the regulation of estrogen synthesis enzymes including 17β-HSD and 3β-HSD. These findings imply that IL-4 is a key enzyme not only for Th2 type immune reactions but also for tumor cell growth itself in human breast cancer.
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Interleukin-4 (IL-4) is a pleiotropic cytokine that exerts biological activities on various cells. It was first identified for its ability to induce B cell proliferation and differentiation[1]. Important functions of IL-4 include B cell growth and immunoglobulin expression, T cell regulation, the generation of cytotoxic effector cells, and growth and differentiation effects on hematopoetic cells[2-5]. Recent accumulated data have shown that IL-4 has potent anti-tumor activity against various types of carcinomas both *in vitro* and *in vivo*. This report summarizes the functions of IL-4, particularly its anti-tumoral activity, in human carcinomas including breast cancer.

Source of IL-4

The main source of IL-4 is a subset of helper T cells (TH2) that also produce IL-5 and IL-6[6]. This subset is clearly defined in mice and provides the most efficient helper activity for B cells. There is, however, no clear-cut evidence for human equivalents of the murine TH1 (IL-2 and IFN-γ producing) and TH2 (IL-4 and IL-5 producing) T cell clones[7]. IL-4 mRNA is detected in less than 5% of activated T cells[8]. However, CD4+T cells infiltrating skin lesions of the patients with atopic dermatitis have been shown to produce high levels of IL-4[9]. Cells other than T lymphocytes, in particular mast cell/basophils and bone marrow stromal cells also produce IL-4[10].

Expression of IL-4 Receptor

IL-4 receptor (IL4-R) expression is detected on most T cells, B cells, mast cells, macrophages, cells of erythroid and myeloid lineage, fibroblasts, and most epithelial cells[11-13], but the receptor number per cell is generally low. The cDNA of IL-4R codes for a single chain transmembrane glycoprotein 140 KD polypeptide belonging to the hematopoietin receptor super family[14-16]. IL-4R is also highly expressed in a wide variety of human tumors of epithelial origin including lung[17], ovary[18], kidney[19, 20], and breast[18, 21] compared with normal cells. These findings indicats that increased expression of IL-4R is involved in the process of tumorigenesis. Approximately 30% of breast tumors have elevated expression of IL-4R [22]. Subsequent analyses indicated that loss or down-regulation of IL-4R could contribute tumor progression via an effect on cell growth and differentiation[23,24]. IL-4 signaling is inhibited by binding of IL-4 to the high-affinity IL-4 receptor alpha-chain and subsequent interaction with the common gamma-chain. Soluble forms of the extracellular domain of the alpha-chain (sIL-4R)

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Abbreviation:
IL, Interleukin; TNF, Tumor necrosis factor; IFN, Interferon; TCR, T cell receptor; TGF, Transforming growth factor; IGF, Insulin-like growth factor; IRS, Insulin receptor substrate; ICAM, Intercellular adhesion molecule; VCAM, Vascular cell adhesion molecule; ELAM, E-selectin; VEGF, Vascular endothelial cell growth factor; HSD, Hydroxysteroid dehydrogenase

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were showed to be present in biological fluids and inhibit IL-4 activity by forming IL-4/sIL-4R complex[25].Human recombinant sIL-4R induced the formation of IL-4/sIL-4R complex in the supernatant of activated T cells in a dose-dependent manner[26]. The production of human sIL-4R by T cells is regulated IL-4 and IFN-gamma and requires the activity of metalloproteinases[27].

Biological Effect of IL-4

IL-4 was first identified as a B cell growth factor (BCGF)[1] because of its ability to promote B cell proliferation. In addition, IL-4 can induce resting B cells to increase their expression of class II major histocompatibility complex (MHC) molecules and low-affinity receptors for the Fc portion of IgE (FcεR). Moreover, IL-4 plays crucial roles in immunoglobulin isotype regulation, not only by inducing the production of the IgG1 isotype in mitogen and T cell stimulated cultures, but also by inducing high IgE levels[2-5]. IL-4 is well konwn to exert various functions on T cells and B cells. Both murine[28] and human[29] thymocytes proliferate in response to IL-4. The proliferative capability of these cells is independent of IL-2. IL-4 can also stimulate the generation of antigen-specific cytotoxic T cells[30, 31]. IL-4 is essential for in vitro growth of mucosal and connective tissue mast cells and may exert an autocrine effect, since it is produced by several transformed and non-transformed mast cell lines[32]. IL-4 also enhances the antigen presenting ability and anti-tumor activity of macrophages and acts on many hemopoietic cell lineages, including immature erythroid, myelomonocytic and megakaryocytic precursors[33]. (Fig 1).

Activity on Breast Cancer Cells

Transfection of the IL-4 gene in tumor cell lines[34-36] and treatment of tumor cells with IL-4 have demonstrated that IL-4 has potent anti-tumor activity against various types of tumors including renal carcinoma, lung carcinoma, thyroid carcinoma, and breast carcinoma[21,37-40,48]. Tepper *et al*[36]. have shown that IL-4 tansfected malignant tumor cell lines (plasmacytoma, breast adenocarcinoma) elicited a potent antitumor response when injected into mice. This antitumor response was observed in nu/nu mice, which are devoid of T cells, and bg/bg mice, which are devoid of NK cells, indicating that T and NK cells are not necessary for the effect of IL-4. Histologic examination of the subcutaneous inoculation site of IL-4 producing tumor cells showed infiltrating macrophages and eosinophils which are hypothesized to mediate the anti-tumor effect of IL-4. The action of IL-4 is clearly dose-dependent, with complete eradication of tumor requiring highly localized concentration of cytokines. The reversal of IL-4-induced tumor cytotoxicity by the in vivo administration of ant-IL-

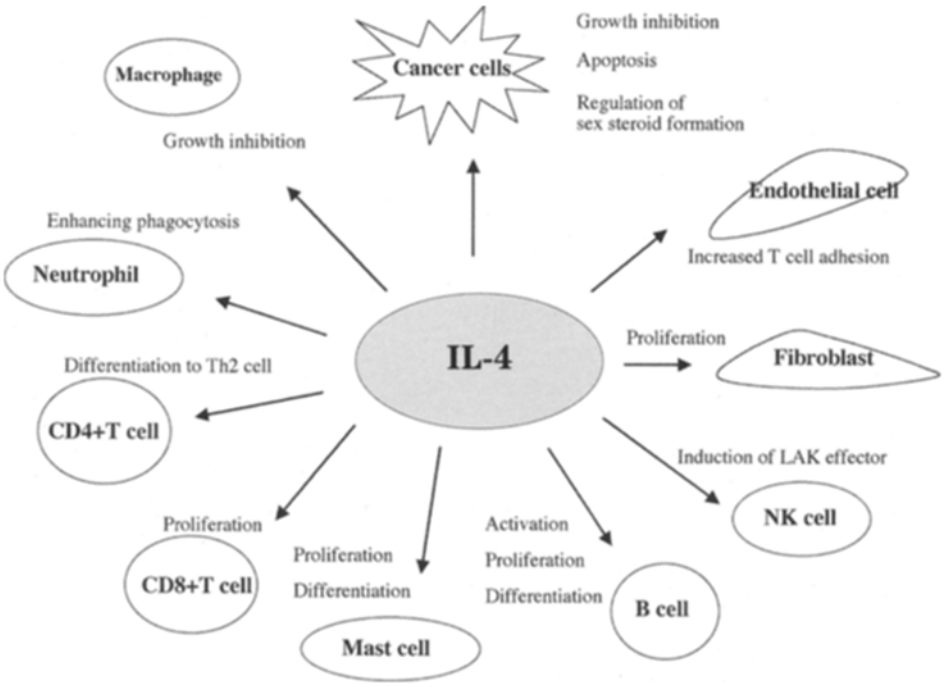

Fig 1. Biological activity of IL-4.

Table 1. IL-4 and Breast Cancer

		Cell lines	Remarks
in vitro	Klara T ('91)[40]	MDA-MB-330	Modulation of TNF-dependent anti-tumor response
	Toi M ('92)[21]	MCF-7 WT, MCF-7r MDA-MB-231, 468	Additive cell growth inhibition with TAM and TGF-β1
	Blais Y ('96)[37]	ZR-75-1, T-47D	Inhibition of estrogen-induced breast cancer cell proliferation and induction of GCDFP-45
	Gooch JL ('98)[41]	MCF-7, MDA-MB-231	Growth inhibition induced by apoptosis
in vitro	Tepper RI ('89)[36]	K485	Growth inhibition mediated by infiltrated inflammatory cells
	Pericle F ('94)[43]	TS/A-pc	Th2 memory in growth inhibition
	Allione A ('94)[44]	TS/A-pc	Protection 80-100% of mice immunized with replicating tumor cells by the induction of IL-4
	Musiani P ('96)[45]	TS/A	Rejection of IL-4 producing TSA cells by neutrophils and CD8+lymphocytes
	Maeda T ('99)[46]	OCUB-M	No tumor formation in IL-4 producing cell *in vivo*
	Pacor S ('99)[47]	TS/A	Paracrine effect of IL-4 induced growth inhibition *in vivo*

4 antibody provides further evidence for the action of IL-4 as a secreted factor. An additive antiproliferative effect of IL-4 with other cytokines has also been reported. The enhancement of the cytotoxic effect of tumor necrosis factor (TNF)-α by IL-4 was showed in breast carcinoma cells (MDA-MB-330) in a dose-dependent manner. This effect was also observed in human epidermoid carcinoma cells and human histiocytic lymphoma cells[40]. Combination treatment with IL-4 and other growth inhibitors, such as TGF-β1 and tamoxifen, showed an additive growth inhibitory effect in breast carcinoma cell lines. IL-4 inhibited 90% of the 17β-estradiol –stimulated growth of MCF-7 WT cells without a change in estrogen receptor expression. Less inhibition of growth by IL-4 occurred when estrogens were absent. The hormone independent cell lines MCF-7r and MDA-MB-231 were inhibited by IL-4 and TGF-β1[21]. Several lines of experimentation have provided evidence that the anti-tumor activity initiated by localized IL-4 secretion may be mainly due to the rapid influx of host effector cells capable of mediating tumor cytotoxicity directly or indirectly through other tumoricidal cytokines[36]. However, recently several investigators have elucidated another mechanism of the anti-tumor effect of IL-4, namely that IL-4 can induce apoptosis in breast cancer cell lines. Insulin-like growth factor (IGF)-1 and IL-4 share a common signaling pathway via the insulin receptor substrate (IRS)-1 molecule. IL-4 had no effect on IGF-1 stimulated proliferation, which is known to inhibit apoptosis, in MCF-7 and MDA-MB-231 cell lines. IGF-1 reversed IL-4 induced growth inhibition, suggesting that the mechanism of IL-4 induced growth inhibition in these cells is the induction of apoptosis[41]. These findings underlie the importance of the balance between IGF-1 and IL-4 for the induction of apoptosis in breast cancer cells. For instance, breast cancer cells that have became hormone resistant are likely to enhance IGF-1 production. Although little is yet known about IL-4 expression in hormone resistant tumors an IGF-1 dominant, IL-4 recessive status might be achieved in such tumors. It is known that E2 and insulin induce polyamine transport in breast cancer, such as the ZR-75-1 cell lines[42]. IL-4 is also characterized to be a potent inducer of polyamine transport. IL-4 caused a marked increase of uptake of radiolabeled spermidine with 80 pg/ml ED50. Interferon (IFN)-γ is reported to mimic the IL-4 induced spermidine uptake. This is an interesting aspect of IL-4 considering its biological function. *In vivo* and *vitro* studies of breast cancer are shown in Table 1.

Effects on the Endothelium

IL-4 mediating expression of adhesion molecules in endothelial cells may be an important mechanism by which IL-4 promotes the selective transmigration of eosinophils into tissues. IL-4 has been to shown to stimulate, alone or synergistically with IL1 or TNF-α, the induction of VCAM-1

Table 2. Phase II Clinical Traial of rIL-4 in Patients with Solid Tumor

	SOG[54]	UCCRC[55]	UC[56]	CHNMC[57]
Tumor Type	advanced melanoma	NSCLC	advanced RCC	melanoma advanced RCC
No. of Pt	36	63	19	49
Dose	5 μg/kg	0.25 or 1.0 μg/kg	1.0 μg/kg	
Route and frequency	s.c., day 1-28 35 d cycle	s.c. 3 times/week	s.c. 3 times/week	i.v.
Response	CR 1, NC 2	PR 1, NC 9	no response	CR 1
Toxicity	nausea, vomiting, diarrhea, malaise, fatigue, edema, headache, myalgias, arthralgias, fever, chill, liver dysfunction	fatigue, fever, vomiting, dyspnea, anorexia, duodenal ulcer	fever, fatigue, myalgias, arthralgias, nausea, anorexia gastrointestinal bleeding	fever, malaise, nasal congestion, diarrhea, cardiac ischemia, gastrointestinal bleeding

SOG, Southwest Oncology Group; UCCRC, University of Chicago Cancer Research Center; UC, University of Chicago; CHNMC; City of Hope National Medical Center; NSCLC, Non-small cell lung cancer; RCC, Renal cell carcinoma.

on human endothelial cells, while ICAM-1 and ELAM-1, which promote the binding of all granulocyte subtypes to endothelium[48], are unaffected by IL-4[49]. IL-4 is a potent regulator of angiogenesis. Interestingly, IL-4 behave bimodally with regard to endothelial cell growth. In lower concentrations at picomolar levels, IL-4 stimulates proliferation and migration of endothelial cells and also can promote in vivo neovascularization. On the other hand, in higher nanomolar concentrations, IL-4 inhibits endothelial cell growth and migration. IL-4 blocked the induction of corneal neovascularization in rat by basic fibroblast growth factor[50]. Furthermore, it was reported that IL-4 down-regulates the expression of one VEGF receptor, VEGF-R2, in an experiment using mouse endothelial cells. The local anti-angiogenic activity of IL-4 could account for the decrease in tumor vessel density observed in IL-4 secreting gliomas[51]. On the other hand, in our clinical analysis of primary breast cancer, we failed to demonstrate a significant correlation between IL-4 levels in tumor tissues and angiogenic grade, which might reflect the bi-functional role of IL-4.

Hormone and IL-4

There is evidence suggesting that local intracrine formation of sex steroids from inactive precursors secreted by the adrenal gland plays a crucial role in the regulation of growth and function of peripheral target tissues, including breast. 17β-HSD and 3β-HSD activities represent critical sites of control and regulation of sex steroid formation and/or inactivation in human breast cancer cells. Turgeon C *et al*[52] investigated the potential effects of IL-4 and IL-6 on 17β-HSD activity in ZR-75-1 and T-47D human breast cancer cell lines, and showed that IL-4 and IL-6 regulate the 17β-HSD activity, whereas IL-4, but not IL-6, may induce 3β-HSD activity in these two cell lines. Interestingly, IL-4 increased oxidative 17β-HSD activity, but significantly decreased reductive 17β-HSD activity in low concentrations (20 pM-100 pM) in ZR-75-1 cells. This enzymatic modulation elicited a marked decrease of E2 formation. In contrast, in T-47D cells, IL-4 increased the formation of E2, which suggests that the regulatory mechanism of 17β-HSD activity by IL-4 differs among cell lines, and perhaps among individual tumors. Genetic expression of 3β-HSD type 1, which is essential for the biosynthesis of active estrogens and androgens, was markedly induced by picomolar levels of IL-4 in various types of cultured breast cancer cells including ZR-75-1, T-47D, and ER-negative MDA-MB-231 cells, and normal mammary epithelium. It was further found that IL-4 can enhance the DNA-binding activity of Stat 6 in these cells, and the Stat6 activated by IL-4 can bind to the promoter region of the 3β-HSD type 1 gene[53]. According to our recent analysis of the intra-factorial correlation among cytokines including IL-4, chemokines and

growth factors in human breast tumor tissues, IL-4 is frequently overexpressed and its expression is significantly associated with other Th-2 type cytokines including IL-10. In addition, intratumoral IL-4 levels tend to be negatively correlated with the level of hormone receptor, which seems to indicate that IL-4 plays important roles in hormone-dependent and -independent growth of breast cancer.

Therapeutic Approaches

Accumulated data supports a potential clinical use of IL-4. Phase I/II clinical trials of IL-4 therapy in patients with solid tumors are already underway. Some of these studies are summarized in Table 2[54-57]. In some studies, IL-4 is being given subcutaneously to patients with renal cell carcinoma, melanoma, and non-small cell lung carcinoma. The doses rages from 0.25-5.0μ g/kg. Clinical trials in patients with breast cancer have not been reported yet. IL-4 can induce serious adverse effects, including fever, nausea, diarrhea, anorexia, fatigue, cardiac ischemia and gastrointestinal bleeding. Although no significant response against solid tumors has been reported to date, several novel approaches focusing on IL-4 related cell growth inhibition and apoptosis are now under investigation.

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