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Interleukin-6 regulates expression of estrogen receptors in human colorectal cancer and acute T-cell leukemia cells

Yue Liu[1] and Zongbing You[1,2,3,4,5,6*]

[1]Department of Structural & Cellular Biology, Tulane University, New Orleans, Louisiana, USA; [2]Department of Orthopaedic Surgery, Tulane University, New Orleans, Louisiana, USA; [3]Tulane Cancer Center and Louisiana Cancer Research Consortium, Tulane University, New Orleans, Louisiana, USA; [4]Tulane Center for Stem Cell Research and Regenerative Medicine, Tulane University, New Orleans, Louisiana, USA; [5]Tulane Center for Aging, Tulane University, New Orleans, Louisiana, USA; [6]Tulane Center of Excellence in Sex-Based Biology & Medicine, Tulane University, New Orleans, Louisiana, USA.
***Correspondence:** zyou@tulane.edu; Tel.: +1-504-988-0467.
Present address: Yue Liu, Laboratory of Tumor Biology, Academy of Bio-Medicine Research, Hubei University of Medicine, Shiyan, Hubei, China.

ABSTRACT

Interleukin-6 (IL-6) is a pleiotropic cytokine involved in inflammation, immune regulation, and tumor progression. Estrogen receptors (ERs), particularly ERα and ERβ, play distinct roles in modulating immune responses and cancer development, yet their regulation by IL-6 in different cancer types remains incompletely understood. In this study, we investigated the effects of IL-6 on expression of ERα and ERβ in human colorectal cancer cells (HCT116) and acute T-cell leukemia cells (Jurkat). The cells were treated with recombinant human IL-6 (at a dosage of 100 ng/ml) for up to 24 hours, and changes in ER expression were assessed using Western blot analysis. We found that IL-6 treatment did not significantly alter ERα or ERβ levels in HCT116 cells, though a non-significant upward trend in ERβ expression was observed. In contrast, Jurkat cells exhibited a statistically significant increase in ERβ expression at 8 and 24 hours after IL-6 stimulation, while ERα expression remained unchanged. These findings suggest a cell-type-specific regulatory role of IL-6 in modulating estrogen receptor expression. The selective upregulation of ERβ in Jurkat cells implies a potential IL-6-ERβ signaling axis in T-cell leukemia, which may contribute to inflammation-driven leukemogenesis and warrants further mechanistic investigation.

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1. Introduction

Autoimmune diseases and cancers remain as the leading causes of morbidity and mortality worldwide, with autoimmune disorders alone affecting approximately 5-10% of the global population and substantially impairing quality of life [1-3]. A growing body of research highlights the central role of cytokine signaling dysregulation in both autoimmune pathogenesis and tumor progression. Among these, interleukin-6 (IL-6) is a pleiotropic inflammatory cytokine that contributes to chronic inflammation, immune imbalance, and tissue remodeling in diseases such as rheumatoid arthritis, inflammatory bowel disease, and various cancers [4]. In parallel, estrogen receptors (ERs), especially ERα and ERβ, have emerged as key immunomodulators. While ERα is commonly associated with pro-inflammatory responses, ERβ has been shown to exert anti-inflammatory effects in both autoimmune and oncological contexts [5,6]. IL-6 is a pro-inflammatory cytokine that plays a crucial role in tumor microenvironment and immune modulation [7,8]. IL-6 primarily exerts its role in cell proliferation, cell differentiation, and inflammation through Janus kinase-signal transducer and activator of transcription 3 (JAK/STAT3) pathway and/or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ƙB) signaling pathway [8]. There are many studies illustrating that overexpression of IL-6 can promote pathogenesis of multiple cancers, including breast cancer, lung cancer, and colorectal cancer [9].

There are two IL-6 signaling pathways: classical signaling and trans-signaling pathways. IL-6 elicits its effects by engaging its receptors IL-6Rα and gp130. In the classical signaling pathway, binding of IL-6 with IL-6Rα induces a conformational change of this receptor complex. The conformational change recruits gp130, leading to the formation of a hexameric sig-

naling complex, which then initiates intracellular signaling through downstream signaling cascades, including the JAK/STAT, mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), and phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathways. The trans-signaling pathway allows IL-6 to exert its effects on cells that lack the membrane-bound IL-6 receptor (mIL-6R). Instead of the mIL-6R, a soluble form of IL-6 receptor exerts its ligand effects. After formation of IL-6-sIL-6R in the extracellular space, it binds to gp130 expressed on the cell membrane. The complex is then guided to interact with cytoplasmic gp130, triggering the final signaling pathway that leads to the phosphorylation of JAK/STAT, activating the pathway [10].

Estrogen receptor α (ERα) and estrogen receptor β (ERβ) are two primary subtypes of estrogen receptors, exhibiting distinct expression patterns and functions in various tissues. The regulatory relationship between IL-6 and ERα appears to be context-dependent, with evidence supporting both upregulation and downregulation under different physiological and pathological conditions. Notably, most reports of ERα downregulation occur at the transcriptional level, particularly in studies focusing on endocrine resistance, dysregulated inflammation, or immune imbalance [11-13]. In contrast, studies describing IL-6-induced ERα up-regulation are relatively limited and often involve non-transcriptional mechanisms such as post-translational modifications (e.g., phosphorylation) [14-16]. These findings emphasized the significance of understanding the microenvironmental and signaling contexts that influence ERα regulation.

ERβ is the predominant ER type in colorectal tissue and lung cancer. It also plays essential roles in maintaining the intestinal barrier, regulating epithelial homeostasis, and combating inflammation. In an inflammatory environment, expression of ERβ may be inhibited, thereby compromising its protective function [17]. Some studies have shown that mice with ERβ knockout background developed more severe colitis compared to the wild-type mice [18,19]. Studies about ERβ-IL6 regulation have been limited in recent years. It is possible that how IL-6 regulates ERα may inspire a similar understanding of ERβ regulation by IL-6.

Although ERβ is the predominant estrogen receptor isoform in colonic epithelium, its expression is not static and can be modulated by various physiological and pathological stimuli. Hormonal fluctuations, oxidative stress, and environmental factors have all been implicated in altering ERβ expression across multiple tissues [20,21]. Recent evidence suggests that inflammatory mediators, such as IL-6, IL-1β, and IL-17A, not only directly contribute to the pathogenesis of colorectal disease but may also interfere with nuclear receptor signaling [22]. These findings suggest that ERβ expression may be sensitive to inflammation-associated cytokine signaling, particularly in chronically inflamed environments such as colorectal tumors or colitis-associated neoplasia.

Several studies focusing on inflammatory bowel disease (IBD) showed that inflammatory mediators likely suppress ERβ expression in intestinal macrophages, contributing to the progression of IBD lesions. For example, a recent study on colorectal cancer showed that expression of ERβ in colonic epithelial cells were downregulated after IL-6 treatment. Caco-2 cells were used for culturing intestinal epithelial cells [18]; the choice of this cell type may facilitate further investigation of the complex relationship between the regulatory axis linking IL-6 and ERβ in IBD.

However, it has to be mentioned that the majority of the research concerning the connection between IL-6 and estrogen receptor mainly focuses on breast cancer, endometrial cancer, and lung cancer, showing that IL-6 up-regulates ERβ expression [19,23,24]. For instance, Wang et al have illustrated the IL-6-STAT3-ERβ axis, suggesting that ERβ is one of the target genes in the IL-6 signaling pathway in lung carcinoma [19]. More than that, Huang's work found that activation of ERβ promoted expression of IL-6. They found that in the lung cancer cell model, 17β-estradiol acted on ERβ, leading to upregulation of IL-6, which in turn caused proliferation and invasion of lung cancer cells. The findings help to understand that ERβ is not only regulated by IL-6, but also reversely regulates IL-6, forming a two-way positive feedback loop [25]. Tang et al focused on the physical and functional interaction between the ERβ5 subtype and the IL-6 receptor (IL-6R), showing that ERβ5 can enhance IL-6R-mediated signaling pathways, further promoting proliferation and migration of lung cancer cells [21]. This research showed that in lung cancer, a regulatory feedback loop exists between IL-6 and ERβ, wherein IL-6 promotes ERβ expression via STAT3 signaling, while activated ERβ or its isoforms further potentiate IL-6-driven tumor progression.

The distinct tendencies of ERβ regulation vary across different diseases, which are unpredictable and

complex. This is the subject that our research attempts to validate further. We aim to study the regulation of ERα and ERβ in different cell lines, which may help inform further animal experiments and clinical trials related to bowel diseases or leukemia.

2. Materials and methods

2.1. Cell culture

Human colorectal cancer cell line HCT116 and human acute T-cell leukemia cell line Jurkat were purchased from American Type Culture Collection (Manassas, VA). HCT116 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Genesee Scientific, Morrisville, NC) prepared with 10% fetal bovine serum (FBS, Peak Serum, Wellington, CO) and 100 U/ml penicillin/streptomycin. Jurkat cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Genesee Scientific, Morrisville, NC) supplemented with 10% FBS and 100 U/ml penicillin/streptomycin. Both cell lines were cultured in a thermal cell incubator with 5% CO_2 at 37°C.

2.2 Reagent

Recombinant human IL-6 was purchased from R&D Systems (Minneapolis, MN). The primary antibodies used were: mouse anti-human ERβ monoclonal antibody (sc-53494, Santa Cruz Biotechnology, Dallas, TX), rabbit anti-human ERα monoclonal antibody (cat#SAB5600097, MilliporeSigma, Burlington, MA), and mouse anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibody (#MAB374, MilliporeSigma, Burlington, MA). The secondary antibodies used were: goat anti-mouse monoclonal antibody (D30613-05, LI-COR Bioscience, Linclon, NE), goat-anti-rabbit monoclonal antibody (D20420-06, LI-COR Bioscience, Linclon, NE), and goat-anti-mouse monoclonal antibody (D20427-25, LI-COR Bioscience, Linclon, NE). Each antibody produced a single, specific band at the expected molecular weight, with no cross-reactivity observed.

2.3 Western blot analysis

After the indicated time of treatment, the cells were collected; proteins were extracted using radioimmunoprecipitation assay (RIPA) lysis buffer (50 mM sodium fluoride, 0.5% Igepal CA-630[NP-40], 10mM sodium phosphate, 150 mM sodium chloride, 25mM Tris pH 8.0, 1mM phenylmethylsulphonyl fluoride, 2 mM ethylenediamine-tetraacetic acid [EDTA], 1.2 mM sodium vanadate) supplemented with 1% protease inhibitor cocktail (Sigma-Aldrich). After equal loading in 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels, the proteins were transferred to polyvinylidene difluoride membranes (PVDF) using a Bio-Rad semi-dry transfer machine for 1 hour. The membranes were blocked with 2.5% bovine serum albumin (BSA) in Tris-buffered saline-Tween 20 (TBS-T) buffer (25 mM Tris-HCl, 125 mM NaCl, 0.1% Tween 20) for 0.5 h and then incubated overnight with the indicated primary antibodies. Then, the membranes were incubated with IRDye 800 CW- or IRD-conjugated secondary antibodies for 1 hour. The results were visualized using an Odyssey infrared imager (LICOR Biosciences). GAPDH was probed for loading control. Quantification of the Western blot signals was performed using the image analysis software of the Odyssey infrared imager system.

2.4 Statistical analysis

The greyscale intensities of the Western blot bands were quantified using ImageJ software. The integrated density values of signals of targeted genes were normalized by GAPDH. Statistical analysis was performed using SPSS (v17.0, SPSS Inc., Chicago, IL). Data are presented as mean ± standard deviation (SD). Quantitative data were analyzed using analysis of variance (ANOVA, two-tailed). Statistical significance was reached with a P value less than 0.05.

3. Results

Human colon cancer cell line (HCT116) and human acute T-cell leukemia cell line Jurkat were treated with 100 ng/ml IL-6 for 0, 0.5, 3, 8, and 24 hours (h). Western Blot analysis found that IL-6 treatment did not affect ERα expression in HCT116 cells (Figure 1A-1B). IL-6 treatment slightly increased ERβ expression level in HCT116 cells (Figure 1A), but the increase was not statistically significant (Figure 1C). IL-6 treatment increased ERα expression in Jurkat cells (Figure 2A), but the increase was not statistically significant (Figure 2B). IL-6 treatment increased ERβ expression (Figure 2A), which was statistically significant ($p < 0.05$) at 8 and 24 h after the treatment (Figure 2C).

Figure 1. Effects of IL-6 treatment on ERα and ERβ expression in HCT116 cells. (A) HCT116 cells were treated with 100ng/ml IL-6 for the indicated time; representative Western blot results are presented. (B-C) Quantification of three different Western blot results of (A). * $p < 0.05$.

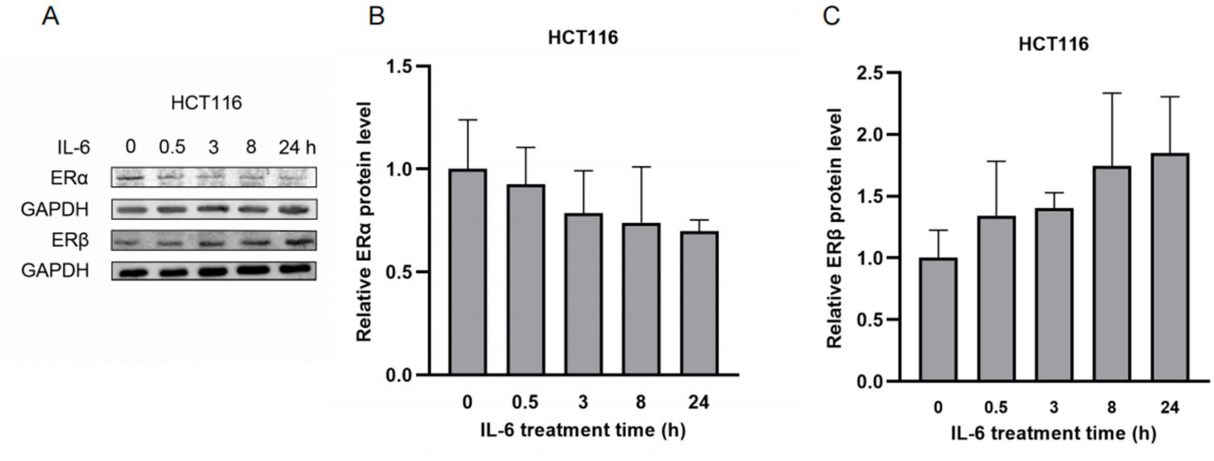
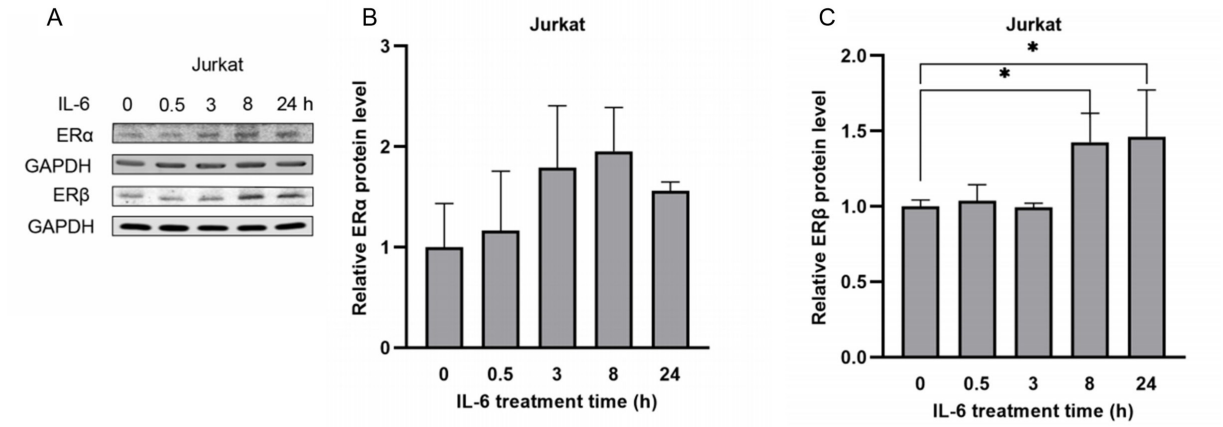


Figure 2. Effects of IL-6 treatment on ERα and ERβ expression in Jurkat cells. (A) Jurkat cells were treated with 100 ng/ml IL-6 for the indicated time; representative Western blot results are presented. (B-C) Quantification of three different Western blot results of (A); * $p < 0.05$.



4. Discussion

In this study, we investigated the regulatory effects of IL-6 on protein expression of estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) in two distinct human cell lines HCT116 (a human colorectal cancer cell line) and Jurkat (a human T-cell leukemia cell line). Our results revealed a significant upregulation of ERβ in Jurkat cells following IL-6 stimulation, particularly at 8 and 24 hours after the treatment. However, in HCT116 cells, although there appeared to be subtle changes in ERα and ERβ expression, the changes did not reach statistical significance. These findings are important for several reasons. First, they highlight the cell-type specificity of IL-6 signaling in modulating ER expression. Most existing literature on IL-6 and ER interaction focuses on epithelial tumors such as breast, endometrial, and lung cancer [19]. Fewer studies have explored these pathways in hematological malignancies such as T-cell leukemia. Our findings contribute to this gap, suggesting that T-cell-derived cancers may possess a distinct cytokine to receptor regulatory axis involving IL-6 and ERβ.

The observed induction of ERβ in Jurkat cells is consistent with previous reports in lung cancer models where IL-6/STAT3 activation leads to ERβ upregulation [6]. Given that ERβ is known to exert

anti-inflammatory and immunomodulatory roles, its induction in leukemia cells may suggests a feedback mechanism to temper IL-6 driven pro-inflammatory signaling, although this remains speculative and warrants further investigation.

In contrast, the absence of significant ER regulation in HCT116 cells may reflect differences in receptor expression, STAT3 activation thresholds, or chromatin accessibility. Alternatively, IL-6 alone may be insufficient to alter ER expression in colorectal cancer cells without additional inflammatory cues, such as IL-17 or TNF-α [1,17]. This points to the importance of microenvironmental context in cytokine and hormone receptor crosstalk. Although our data did not show statistically significant changes in HCT116 cells, the non-significant trends observed in ERα and ERβ expression remain intriguing. With larger sample sizes by increasing the numbers of repeating experiments, longer stimulation times, or combination of cytokine treatment, these trends may reach significance. In this experiment, only one cell line of each type was tested, this might limit generalizability. Confirming these effects in additional cell lines would be a future direction. We used the same IL-6 concentration for both cell lines to allow a direct comparison under uniform conditions, and because this dose is known to robustly activate IL-6 signaling in diverse cells. Future studies could explore dose-response optimization for each cell lines to determine if different doses yield distinct outcomes. Together, our findings suggest that IL-6 can selectively regulate estrogen receptor expression depending on the tumor cell type. Particularly, the IL-6-ERβ axis in T-cell leukemia may represent a novel signaling mechanism with potential relevance to inflammation-driven leukemogenesis and therapeutic targeting. To assess the biological consequences of ER elevation, phenotype assay could be highly recommended.

5. Conclusion

The present study suggests that IL-6 upregulates ERβ expression in Jurkat cells. Given that IL-6 has been found to be enriched in colorectal tumors and prostate cancer, our findings suggest that IL-6 may play a pro-inflammatory role by upregulating ERβ expression.

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